GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

GEOHAB CORE RESEARCH PROJECT: HABs IN BENTHIC SYSTEMS

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GLOBAL ECOLOGY AND OCEANOGRAPHY OF HARMFUL ALGAL BLOOMS

GEOHAB CORE RESEARCH PROJECT: HABs in Benthic Systems

AN INTERNATIONAL PROGRAMME SPONSORED BY THE SCIENTIFIC COMMITTEE ON OCEANIC RESEARCH (SCOR) AND THE INTERGOVERNMENTAL OCEANOGRAPHIC COMMISSION (IOC) OF UNESCO

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THIS REPORT IS BASED ON CONTRIBUTIONS AND DISCUSSIONS BY PARTICIPANTS OF THE GEOHAB OPEN SCIENCE MEETING ON HABS IN BENTHIC SYSTEMS AND MEMBERS OF THE GEOHAB SCIENTIFIC STEERING COMMITTEE

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EXECUTIVE SUMMARY

Harmful events associated with benthic microalgae (BHABs), have been reported more frequently over the last decade including in areas where BHAB genera were hardly known. Of particular concern are the outbreaks of *Gambierdiscus*, a toxic dinoflagellate genus associated to the ciguatera fish poisoning (CFP). The ciguatoxins produced by *Gambierdiscus* are bioaccumulated in reef fishes and are responsible for the most common algal toxin-related illnesses, globally affecting the greatest number of victims and often with significant long-term health effects. Although traditionally considered as a tropical sickness, CFP is also being recently reported on subtropical waters and the Mediterranean Sea, and new species of *Gambierdiscus* have been identified in these areas.

The outbreaks of *Ostreopsis*, another dinoflagellate genus, are exhibiting similar trends. Ostreopsis outbreaks, first reported in the Mediterranean Sea in 1972, have become more frequent and intense. They are associated with faunal damage and human illnesses, including both food intoxication and respiratory problems. Recent reports of *Ostreopsis* blooms already encompass the whole Mediterranean coastal area, and they have recently been detected in Japanese, Russian, French and Spanish Atlantic coastal waters. The scenario of more frequent BHAB events with range extensions to higher latitudes is especially troubling because our knowledge of BHAB species is nascent and, even in developed nations, the regulatory and monitoring infrastructure in most cases is not adequate to protect public health and marine resources.

Upon request from the scientific community studying harmful algal blooms, the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) program—sponsored by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO—initiated a new Core Research Project on Benthic Harmful Algal Blooms to foster research on events caused by benthic microalgae. In June 2010 in Honolulu, Hawaii (USA), an international Open Science Meeting (OSM) took place. There scientists involved in the study of benthic and planktonic microalgae identified the needs and impediments to research on the ecology, oceanography and physiology of BHABs and management of BHAB-related human illnesses and marine resource losses. Those needs included infrastructure development, detection technologies, monitoring tools and strategies, as well as research to generate data for predictive models of environmental conditions that would be conducive to bloom development. The present report outlines the issues addressed by the participants during the OSM. These included the state of the art regarding taxonomy, sampling methods, biogeography, genetic diversity, ecology and ecophysiology of BHABs. The participants also compiled information about current national and regional projects and programmes related to BHABs. The OSM concluded with the identification of research and infrastructure priorities and approaches to be taken in order to improve understanding and prediction of BHABs caused by *Gambierdiscus* and *Ostreopsis*.

Meeting participants developed a "road map" for advancement of the ongoing research on BHABs with the following key questions:

Diversity - How many species are in the BHAB genera? Which ones are toxic?

Biogeography - What is the worldwide distribution and abundance of benthic harmful species?

Sampling - How can BHAB species be sampled effectively and reliably?

Physiology - What are the environmental parameters that affect the physiology of benthic dinoflagellates?

Ecology - What are the main ecological interactions (substrate specificity, trophic links, competition, cooperation) related to benthic dinoflagellates?

Life cycle - What are the life cycles of the benthic dinoflagellates and their possible relationships with the seasonal benthic community composition?

Models - What conceptual and numerical models can be applied specifically to BHABs?

Detection and monitoring technologies - What best suited detection technologies can be developed to support monitoring programs for harmful species and/or their toxins?

Typical Caribbean habitat for benthic harmful algae, Gambierdiscus *and* Ostreopsis *(NOAA).*

LIST OF ACRONYMS

BHABs	Benthic Harmful Algal Blooms
CFP	Ciguatera Fish Poisoning
CRP	Core Research Project
CTX	Ciguatoxin
GEOHAB	Global Ecology and Oceanography of Harmful Algal Blooms programme
GTX	Gambiertoxin
HAB	Harmful Algal Bloom
IOC	Intergovernmental Oceanographic Commission
LC-MS	Liquid Chromatography-Mass Spectrometry
LSU	Large SubUnit
MTX	Maitotoxin
OSM	Open Science Meeting
PLTX	Palytoxin
rDNA	Ribosomal Deoxyribonucleic Acid
SCOR	Scientific Committee on Oceanic Research
UNESCO	United Nations Environmental, Scientific and Cultural Organization

I. INTRODUCTION

The Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) programme was initiated in 1999 by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO, to foster research on the ecological and oceanographic mechanisms underlying the population dynamics of harmful algal blooms (HABs). The ultimate goal of the GEOHAB research programme is to allow the development of observation systems and models that will enable prediction of HABs, thereby reducing their impact on the health of humans and marine organisms as well as their economic impacts.

To achieve its objective, the GEOHAB Science Plan (GEOHAB, 2001) and the GEOHAB Implementation Plan (GEOHAB, 2003) specified, among other activities, the formation of Core Research Projects (CRPs) to address comparative, integrative, multi-faceted and international research on HABs. In the beginning, four CRPs related to four ecosystems types (fjords and coastal embayments, upwelling, eutrophic, and stratified systems) were established. These CRPs were initiated through focused open science meetings (OSMs) with a wide international participation. At the OSMs, scientists discussed the state of the art on the harmful events developed in each of the systems and highlighted the main questions that could be addressed in subsequent research efforts. The main outcomes from these meetings were research plans for each CRP, available in hard copy and electronic versions (see www.geohab.info).

GEOHAB was designed to be open to new initiatives that HAB researchers would identify during the life of the programme. This was the case for harmful events caused by benthic microalgae; it was evident mid-way through the programme that it was important to start a new CRP within GEOHAB, for several reasons (Zingone et al., 2012).

First, BHABs appear to be impacting a wider area in recent years. Whether this is due to increased awareness or to an actual increase in bloom frequency and intensity is still under debate. Indeed, it seems that certain harmful benthic species have been identified in new areas. Examples include *Ostreopsis* spp. along the Mediterranean, Australian and Japanese coasts (e.g. Vila et al., 2001; Shears and Ross, 2009; Omura et al., 2010; Parsons et al., 2012) and recent records of *Gambierdiscus* sp. in the Mediterranean Sea (Aligizaki and Nikolaidis, 2008) and Micronesia and Macaronesia (Pérez-Arellano et al., 2005). Bloom intensity also seems to have increased at some places, where outbreaks have attracted public attention to this phenomenon. For instance, in the Mediterranean Sea, benthic animals were visibly harmed during an *Ostreopsis* cf. *ovata* bloom in the Tyrrhenian Sea (Sansoni et al., 2003), while in the Ligurian Sea 200 people were hospitalized in 2003 due to health problems ascribed to the presence of *O.* cf. *ovata* toxins carried by aerosols from the bloom or released directly into the affected waters.

Second, the increased attention to the BHAB phenomenon highlighted the disparity in our knowledge of the ecology of the species associated with the microphytobenthos compared to phytoplanktonic species. In view of the paucity of scientists studying the microphytobenthos, several phytoplanktologists have expanded their research scope to include benthic microalgae. While the morphology, taxonomy and phylogeny issues concerning benthic and planktonic HAB species may not differ greatly, the two groups are clearly distinct regarding their habitats, relationships with the physical and biological environments, and spatial and temporal scales of variability. The intrinsic characteristics of the planktonic and benthic HABs also require that different approaches and methodologies be applied in their study. Some of these, especially sampling methods, are far from being optimized. In this respect, some ecologists quite familiar with plankton may feel naive, and in some ways beginners, when dealing with benthic microalgae.

A third driver for the new CRP on BHABs is it provides a good opportunity to share the collective experience and expertise accumulated over the years on tropical and subtropical benthic HABs, namely those related to *Gambierdiscus* spp. This experience can be particularly useful in view of the recent expansion of BHAB problems to temperate areas. These recent discoveries have brought new researchers into the field, with a considerable increase in related publications (Shears and Ross, 2009). The establishment of an international program that can coordinate information and data sharing of different science communities, many of which are geographically separated, is the objective of the BHAB CRP and fits well within the main goals of GEOHAB.

The purpose of this document is to describe the main topics that were highlighted during the OSM regarding BHABs (distribution, abundance, ecophysiology, taxonomy and sampling). This document also compiles the suggestions, questions and general objectives to be pursued within the GEOHAB CRP – HABs in Benthic Systems in the coming years. Finally, this document serves as an invitation to individuals who could not participate in the BHABs OSM to join these activities and contribute their talents and expertise to closing the information gaps within the next decade.

The report combines the contributions of the participants through communications and/or discussion sessions at the OSM (see programme in Appendix I) and a description of the main topics highlighted at the meeting. During the elapsed time since the OSM until the final edition of the report, new results have been published. Some of these results have also been included in the Report, which however should not be considered as an exhaustive revision of the state-of-the-art on BHABs. The organizing committee acknowledges here the contributions from scientists who participated in the OSM (see Appendix II) and helped develop the ideas contained herein. The GEOHAB Science Steering Committee is also grateful to the organizations that contributed financially to the meeting, specifically the Intergovernmental Oceanographic Commission (IOC), Scientific Committee on Oceanic Research (SCOR), and U.S. National Science Foundation (Division of Ocean Sciences grants OCE-0938349 and OCE-0813697 to SCOR).

The Smithsonian National Museum of Natural History, Caribbean Coral Reef Ecosystem's laboratory at Carrie Bow Cay, Belize, Central America, the type location of four Gambierdiscus *species (NOAA).*

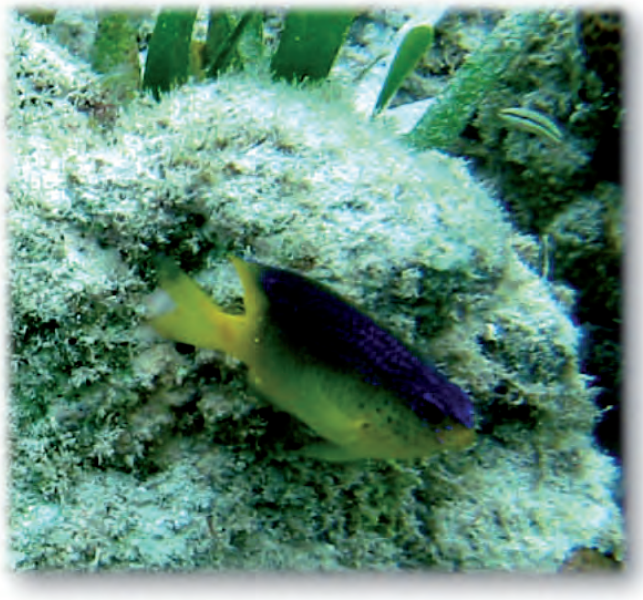
II. THE GEOHAB APPROACH TO THE STUDY OF BHABs

The overall strategy of GEOHAB is to apply a comparative research approach (GEOHAB, 2001; Anderson et al., 2005). The comparative method assembles observations and hypotheses needed for scientific inference by recognizing naturally occurring patterns, and temporal and spatial variations in existing conditions and phenomena. A comprehensive understanding of the population dynamics of BHABs may be reached by comparing studies on the ecophysiology, biology and the spatio-temporal distribution of the main taxa involved in the harmful outbreaks. The results from field and laboratory studies should be combined, if possible, with the application of models of comparable ecosystem types.

In addition to this general GEOHAB approach, the study of BHABs incorporates a new perspective from benthic ecology. Intrinsically, benthic organisms depend strictly on the substrate, are extremely patchy in distribution and vary greatly in abundance. Patchiness poses a major problem for sampling design and execution. Differences in sampling methodologies and other technical limitations may explain some of the discrepancies found in the literature regarding the abundance, ecology and population dynamics of benthic dinoflagellates. This patchiness likely also reflects the importance of local habitat characteristics (e.g. wave and light exposure, topography, substrate) for the establishment of benthic assemblages and links (competition, predation) within them. Consequently, variability may be quite high at small, local scales, while patterns at larger scales may be more predictable.

The adequate merging of the benthic ecological perspective with the GEOHAB comparative approach will assist in addressing the main steps that will contribute to the understanding and prediction of harmful microphytobenthic events. The CRP approach will be to:

- determine the primary physiological, genetic, environmental or behavioral processes that regulate cellular growth and toxicity
- define common characteristics, including the groupings of harmful species from similar habitat types and identification of functional groups
- identify the important physical and chemical factors that control abundance and distribution over appropriate temporal and spatial scales
- inform conceptual and numerical models that help to predict BHAB events
- develop and validate innovative technologies for detailed and extensive monitoring and establish real-time observation platforms.



*Strong site fidelity is a well-known behavior of damselfish. This fidelity likely contributes to the bioconcentration of algal toxins in the marine food web. Pictured here is a juvenile Beaugregory (*Stegastes leucostictus*), typical throughout the Caribbean (NOAA).*

III. AN OVERVIEW OF BHABs

A. General Presentation of HABs in Benthic Systems

A1. Overview of BHAB Problems and BHAB Taxa

Ciguatera fish poisoning (CFP) is a food-borne illness affecting humans, marine resources and local economies worldwide. Humans are affected by this illness after eating reef fish containing the naturally occurring toxins known collectively as ciguatoxins (CTXs). These toxins are produced by benthic dinoflagellates of the genus *Gambierdiscus*, which are found predominantly in association with macroalgae on coral reefs in tropical waters. Ciguatoxins are lipid-soluble and readily transferred through the food web from algae, to herbivorous fish, to carnivorous fish, and ultimately to humans. Multiple structural forms of the toxins (congeners) with varying toxicities have been identified (Scheuer et al., 1967; Murata et al., 1989).

Gambierdiscus taxonomy was revised in 2009 when four species were added to the genus (Figure 1; Litaker et al., 2009) and most recently a new *Gambierdiscus* species was described by Fraga et al., (2011). Two more groups of isolates whose rDNA sequences fall into distinct clusters (ribotypes) in phylogenetic analyses, and which likely represent new species, have also been recognized (Litaker et al., 2010). There are currently a total of eleven described *Gambierdiscus* species (Table 1).

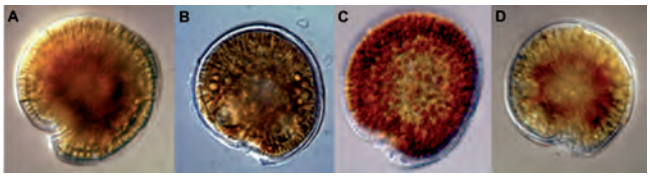


Figure 1. Light micrographs of four newly described Gambierdiscus *species, demonstrating their morphological similarities and supporting the need for molecular methods to discriminate species level differences a)* G. caribaeus*, b)* G. carolinianus*, c)* G. carpenteri*, d)* G. belizeanus *(courtesy of M. Vandersea, NOAA).*

Ostreopsis (Figure 2) is another genus of benthic dinoflagellates that fits within the BHAB definition. Some species produce palytoxin-like toxins that are powerful vasoconstrictors in mammals (Usami et al.,

Table 1. Species of Gambierdiscus *with selected references.*

Species	Geographic Origin	Selected References
Gambierdiscus toxicus Adachi et Fukuyo, 1979	Gambier Islands, French Polynesia, South Pacific Ocean	Adachi and Fukuyo (1979); Litaker et al. (2009)
G. belizeanus Faust, 1995	Belize, Central America, Caribbean Sea	Faust (1995); Litaker et al. (2009)
G. yasumotoi Holmes, 1998	Singapore island of Pulau Hantu, Southeast Asia	Holmes (1998); Litaker et al. (2009)
G. australes Faust et Chinain, *1999*	Australes Archipelago, South Pacific Ocean	Chinain et al. (1999); Litaker et al. (2009)
G. pacificus Chinain et Faust, 1999	Tuamotu Archipelago, South Pacific Ocean	Chinain et al. (1999); Litaker et al. (2009)
G. polynesiensis Chinain et Faust, 1999	Australes and Tuamotu Archipelago, South Pacific Ocean	Chinain et al. (1999); Litaker et al. (2009)
G. carolinianus Litaker et al., 2009	Continental Shelf, North Carolina USA	Litaker et al. (2009)
G. caribaeus Vandersea et al., 2009	Belize, Central America, Caribbean Sea	Litaker et al. (2009)
G. ruetzleri Faust et al., 2009	Belize, Central America, Caribbean Sea	Litaker et al. (2009)
G. carpenteri Kibler et al., 2009	Belize, Central America, Caribbean Sea	Litaker et al. (2009)
G. excentricus Fraga, 2011	Canary Islands, Northeast Atlantic Ocean	Fraga et al. (2011)

1995; Rhodes et al., 2002; Tichadou et al., 2010). Several new toxins have been described for *Ostreopsis* cf. *ovata* from the Mediterranean Sea (Ciminiello et al., 2010; 2012; Rossi et al., 2010), which have been associated with skin irritations to swimmers (Deeds and Schwartz, 2010) and respiratory problems during outbreaks in the Mediterranean Sea (Ciminiello et al., 2008). In cases of extreme exposure, symptoms include vomiting, labored breathing, kidney problems and, in some cases, death (Deeds and Schwartz, 2010). It is not certain if skin irritation and respiratory distress are caused by toxin(s) released into the water or by direct exposure to cells themselves. There is growing evidence that toxins produced by *Ostreopsis* species can be transmitted through the food web to humans eating bottom-feeding fishes, shellfish or sea urchins (Rhodes et al., 2002; Aligizaki et al., 2008). Negative impacts on the benthic macrofauna have also been reported during *Ostreopsis* blooms and include the loss of spines and eventual death of sea urchins and the loss of one or more arms in sea stars (Simoni et al., 2003; Shears and Ross, 2009).

Ostreopsis was considered a monospecific genus for 80 years before Fukuyo (1981) redescribed the type and added two species. Since 1981, seven new species have been described (Table 2). Concerted efforts to revise and clarify the taxonomy of *Ostreopsis* are underway via reexamination of morphological characteristics and genetic characteristics.

The increased numbers of adverse incidences associated with both *Gambierdiscus* and *Ostreopsis* species have generated strong research interests in the last five years, particularly with regard to taxonomy and toxin characterization.

The possibility also exists that toxins produced by certain species of *Coolia* and benthic *Prorocentrum* genera may also play a role in harmful events. Some benthic *Prorocentrum* species have been shown to produce okadaic acid, dinophysis toxins, and fast-acting toxin, which have been implicated in causing diarrhetic shellfish poisoning (DSP) worldwide (Hu et al., 1983; Carlson, 1984; Tindall et al., 1984; Ten-Hage et al., 2000; Faust et al., 2008; An et al., 2010; Skinner et al., 2011). Blooms have additionally been associated with the death of oyster larvae (Wikfors and Smolowitz, 1995).

Coolia tropicalis (reported as *C. monotis*) has been shown to produce the so-called *coolia*toxins (Holmes et al., 1995; Usami et al., 1995; Rhodes et al., 2002).

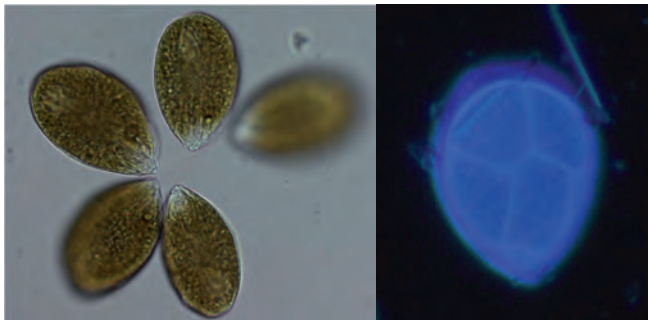


Figure 2. Light microscopy (left; from Barone, 2007, with permission) and epifluorescence image of calcofluor stained (right; courtesy of M. Vila, unpublished) Ostreopsis *cf.* ovata.

Table 2. Species of Ostreopsis *with selected references.*

Species	Geographic Origin	Selected References
Ostreopsis siamensis Schmidt, 1901	Trat Province, Gulf of Thailand	Schmidt (1901); Fukuyo (1980); Penna et al. (2005; 2010); Sato et al. (2011)
Ostreopsis lenticularis Fukuyo, 1980	Gambier Islands and Society Islands, French Polynesia, South Pacific Ocean	Fukuyo (1980); Norris et al. (1985); Larsen and Nguyen (2004)
Ostreopsis ovata Fukuyo, 1980	French Polynesia, New Caledonia and Ryukyu Islands, South and West Pacific Ocean	Fukuyo (1980); Penna et al. (2005; 2010); Sato et al. (2011)
Ostreopsis heptagona, Norris, Bomber et Balech, 1985	Florida Keys, Gulf of Mexico	Norris et al. (1985)
Ostreopsis mascaranensis, Quod 1994	Reunion Island, West Indian Ocean	Quod (1994)
Ostreopsis labens Faust et Morton, 1995	Belize, Central America, Caribbean Sea	Faust and Morton (1995)
Ostreopsis marinus Faust, 1999	Belize, Central America, Caribbean Sea	Faust (1999)
Ostreopsis belizeanus Faust, 1999	Belize, Central America, Caribbean Sea	Faust (1999)
Ostreopsis caribbeanus Faust, 1999	La Parguera, Puerto Rico, Caribbean Sea	Faust (1999)

This toxin is still uncharacterized. Although it was suggested that it might be a yessotoxin, subsequent analyses by mass spectrometry fragmentation have discarded this hypothesis. In most instances any negative effect of these toxins has been largely inferred from the co-occurrence of specific *Prorocentrum* or *Coolia* species with fish kills or outbreaks of DSP. However, the toxins that can be attributed to species in these two genera have yet to be recovered from fish tissues in areas affected by ciguatera (Holmes et al., 1995). The actual causal linkage between the toxins produced by *Prorocentrum* and *Coolia* and the observed effects has not been definitively established yet.

Furthermore, some benthic diatoms, namely *Nitzschia navis-varingica* and *Amphora coffeaeformis*, have been proven to produce domoic acid but their impact has not been assessed and, for the time being, does not seem to represent a pressing problem. However, cyanobacteria might contribute to the origin of CFP toxins and palytoxins based on CFP-like outbreaks in New Caledonia and the Cook Islands associated with the occurrence of *Hydrocoleum*, *Trichodesmium* spp. and *Lyngbia majuscula* (Evans, 2008; Laurent et al., 2008; Kerbrat et al., 2010; 2011).

Given these limited data, the literature on potentially harmful *Prorocentrum*, *Coolia*, benthic diatoms and cyanobacteria will not be reviewed in this document, which will focus on *Gambierdiscus* and *Ostreopsis*. **We propose additional research to determine the relative importance of other BHAB in the coming decade.** Based on the experience from planktonic HABs, it is possible that other benthic genera and/or species previously not considered to be harmful will cause damage through toxin production or other environmental impacts such as mucilage production or anoxia events.

A2. Main Geographic Areas Affected by BHABs

Species of the genus *Gambierdiscus* have a tropical-subtropical distribution and occur in shallow (less than 50 m) coastal and reef habitats globally. This distribution was originally inferred from CFP incidences (Figure 3) because, unlike many HAB species, the toxins and their food web implication on human diets were studied before the taxonomy of the causative species was well understood. Note that this map of CFP incidences reflects the toxic *Gambierdiscus* distribution and likely underrepresents the true range of the whole genus. The first report of a ciguatera event was probably found in a 1525 document concerning an outbreak in Cameroon (Box 1). Recently, CFP was detected in this area (Bienfang et al., 2008).

Current information documents that at least four *Gambierdiscus* species are distributed globally, with the other seven described species found only in the Pacific, Indian or the Atlantic Oceans (including the Mediterranean Sea; Litaker et al., 2010; see also section C1.1).

Ostreopsis is also widely distributed in coastal areas of tropical and warm temperate seas (Figure 4). The phylogeographic study by Penna et al. (2010) suggests that the *O.* cf. *ovata* isolates of the Atlantic and Mediterranean regions may constitute a panmictic population, highly distinct from the Indo-Pacific populations. They also found a clear distinct genotype attributable to the correspondent morphotype of *O.* cf. *siamensis* in the Mediterranean, (see also Parson et al. 2012) while strains identified as *O. lenticularis* and *O. labens* were reported from the Indo-Pacific region.

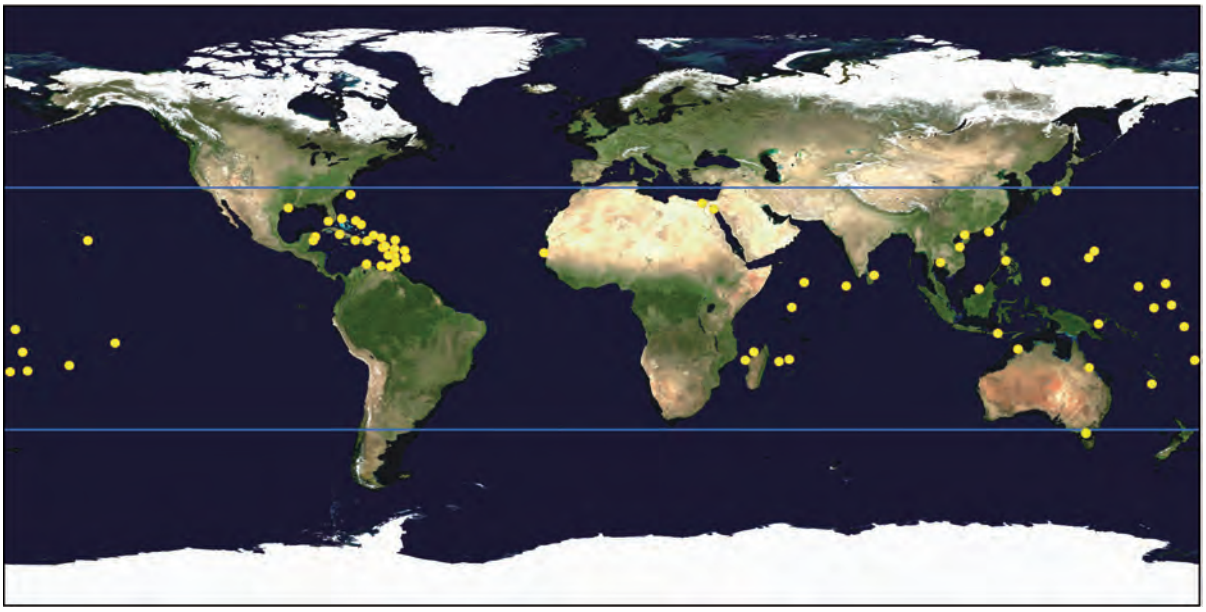
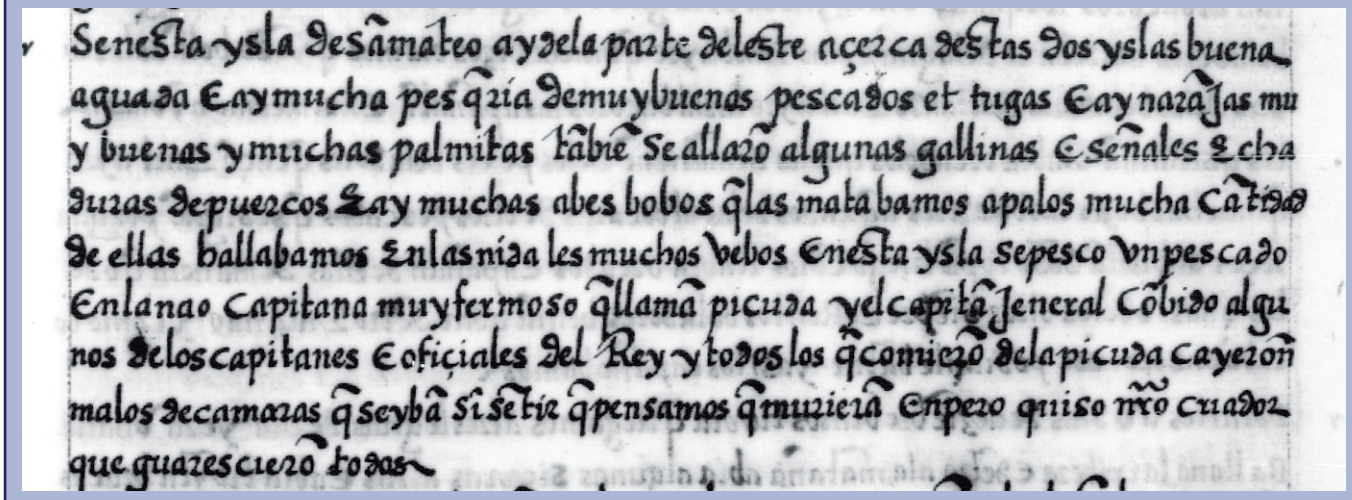


Figure 3. Ciguatera fish poisoning incidences from data updated from Tester et al., 2010. Longitude lines (light blue) are marked at 35º north and 35º south.

Box 1. Spanish manuscript reporting a ciguatera fish poisoning in 1525 in the Gulf of Guinea (Urdaneta, 1580).



"Juan Sebastián Elcano was the first sailor who circumnavegated the world. The selected text describes that during the second trip from Spain to the Pacific Ocean through the Magellan Strait in 1525, the seven ships in the fleet stopped at the Gulf of Guinea. There, the Captain General invited some of the King's officers to a dinner where they were served a very big barracuda. Those who ate the fish were seriously poisoned, suffered diarrhea and lost consciousness. Although they initially recovered, all the intoxicated personnel perished afterwards during the trip, for unknown reasons. This could be the oldest written report of a ciguatera outbreak. Most likely the bad food and living conditions in the ships did not allow them to overcome the effects of the intoxication. Only one of the seven ships reached the Magellan Strait, and Elcano died sailing in the Pacific.

In Spanish, the text says:

"En esta ysla se pescó un pescado en la nao Capitana muy fermoso que se llama picuda y el Capitán General convidó algunos de los capitanes e oficiales del Rey y todos los que comieron de la picuda cayeron malos de cámaras que se iban sin sentir que pensamos que murieran empero quiso nuestro criador que guarescieran todos".

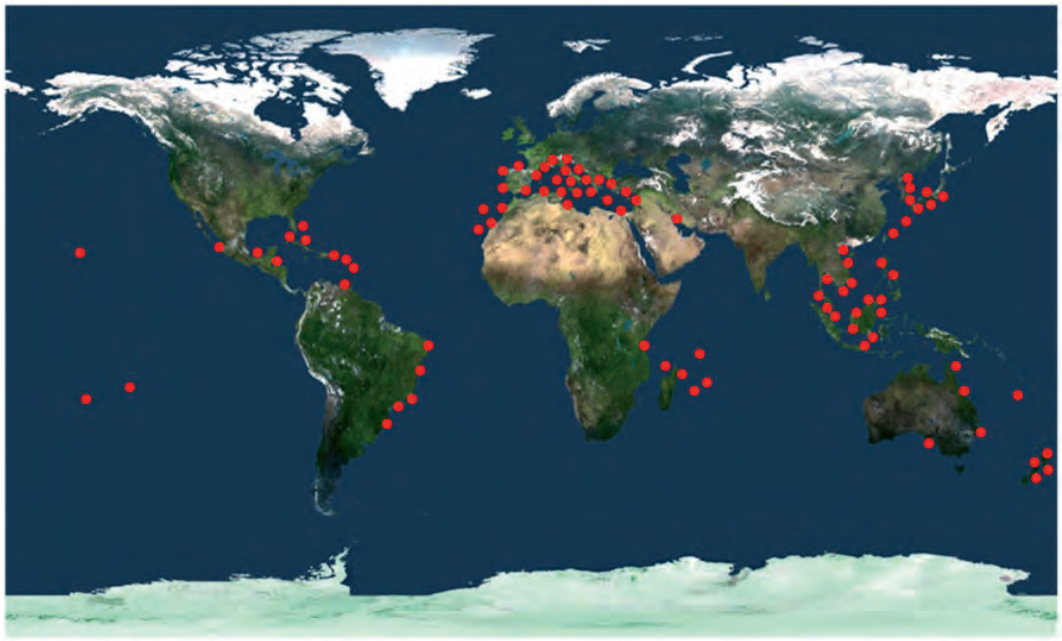


Figure 4. Geographical distribution of the genus Ostreopsis*, redrawn and updated from Rhodes (2010) by A. Zingone.*

Because benthic organisms, including BHAB species, have not been extensively investigated and have extremely patchy distributions, we expect to see these distribution maps change over the course of this CRP. Thus, in Figures 3 and 4, **only a few dots** are found **along African coasts**. This should not be interpreted as the absence of CFP incidence or *Ostreopsis* in the area. Rather, it reflects the lack of relevant data.

At large scale, the occurrence of BHAB species appears to be confined to particular habitats (shallow coastal areas and reefs, Figure 5). However, at a local level the patchiness reflects the importance of habitat characteristics (wave and light exposure, topography, suitable benthic substrate) for the establishment of benthic assemblages as well as the existence of tight links (competition, predation) within them (see sections D3 and D4). Patchiness poses a major problem for the sampling design, and is discussed in more detail in section C3. Sampling methods. Differences in methodologies along with other technical limitations may explain inconsistencies often found in the literature regarding the ecology and population dynamics of benthic dinoflagellates.

The degree to which various environmental factors control the distribution and abundance of individual BHAB species is largely unknown and would be a major focus of the CRP. The population dynamics of phytoplankton and that of harmful algae as well, are known to result from the combination of biological, chemical and physical factors that interplay at a wide variety of spatio-temporal scales (e.g. Chang and Dickey, 2008). Knowing the relevant scales of these forcing factors and magnitude of their effects on the variability of distribution and abundance is especially important (Hales et al., 1999; Llewellyn 2010).

Figure 5. Caribbean fore reef ecosystem, which is typical of the relatively sheltered environments that provide ideal habitat for the growth of BHABs (NOAA).

B. Taxonomy and Genetic Diversity of BHAB Producing Organisms

B1. *Gambierdiscus*

The taxonomy of *Gambierdiscus* species is based on cell size and shape as well as the size and shape of the thecal plates covering the surface of the cell. The first report of a species of *Gambierdiscus* is probably by Silva (1956, p. 359, Plate III, figures 16-18, as *Goniodoma* sp.) from West Africa (Figure 6). Fraga et al. (2011), describing *G. excentricus* from the Canary Islands suggest that Silva's species is conspecific with ribotype I from Belize (Litaker et al., 2009), a sibling species to *G. polynesiensis*. *Gambierdiscus* was formally described by Adachi and Fukuyo (1979) from French Polynesia with *G. toxicus* as the type species. The genus was considered monotypic for almost two decades. However, Tester et al. (2008) and Litaker et al. (2009) provided strong evidence that the original description of *G. toxicus* includes multiple species and therefore propose to designate the material of Adachi and Fukuyo (1979) as the lectotype (Figure 6), and that of Chinain et al. (1999) as an epitype (Figure 7).

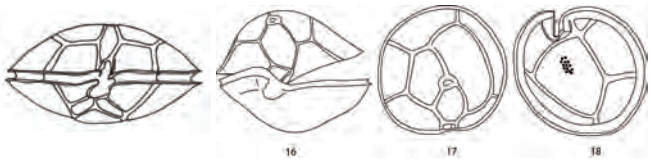


Figure 6. Left: Ventral view of Gambierdiscus toxicus *redrawn from figure 1 of Adachi and Fukuyo (1979), the lectotype. Right: Redrawn view of the* Goniodoma *sp. cell reported from Silva (1956): ventral view (16), epitheca (17) and hypotheca (18); the numbers refer to Silva (1956). This represents the first documented* Gambierdiscus *cell.*

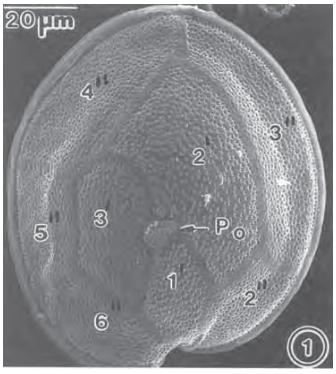


Figure 7. Scanning electron micrograph of the surface epithecal morphology of Gambierdiscus *toxicus (GTT-91). Epithecal view figure 1 from Chinain et al. (1999), the epitype. Republished by permission of Wiley Subscriptions Services, Inc. Copyright (1999) from the Phycological Society of America.*

Most species of *Gambierdiscus*, including the type species *G. toxicus*, are anterior-posteriorly compressed, but two species, namely *G. ruetzleri* and *G. yasumotoi*, differ by having a globular cell shape. The globular species are also distinguished from the anterior-posteriorly compressed species by the size and shape of the 4' and

2' plates (the 1' and 2' plates, respectively, in Litaker et al., 2009), and by differences in the structure of the cingulum and sulcus (Figure 8). Genetically, the globular species are also clearly separated from the anterior-posteriorly compressed species. Litaker et al. (2009) briefly discussed whether these differences are sufficient to warrant the establishment of a separate genus for the globular species, but without drawing any taxonomic conclusion. However, detailed morphological investigations of ten species of *Gambierdiscus*, indicated the most important feature used to discriminate species was the size and shape of the 2'''' and 1p plates (Figure 9, Litaker et al., 2009). The morphological differences among the species were subtle but provided ample features to support a key (Litaker et al., 2009) that has been useful in teaching students to identify *Gambierdiscus* at the species level (Drs. Karen Steidinger and Carmelo Tomas, pers. comm.). By combining rDNA-based molecular data with detailed morphological studies, Litaker et al. (2009) found that phylogenetic analyses based on rDNA data produced distinct species-specific clusters which agreed well with the morphologically defined species. This study included the six previously described species, and description of four new species (Figure 10, Table 1). Litaker et al. (2009) also provided promising perspectives regarding the development of molecular methods for identifying *Gambierdiscus* species. Use of molecular assays would greatly facilitate future studies as species identification based on morphology alone is often dubious (see above). Recently, Fraga et al. (2011) described *G. excentricus* from the Canary Islands (Table 1) and this species is clearly distinct morphologically as well as phylogenetically from previously described species.

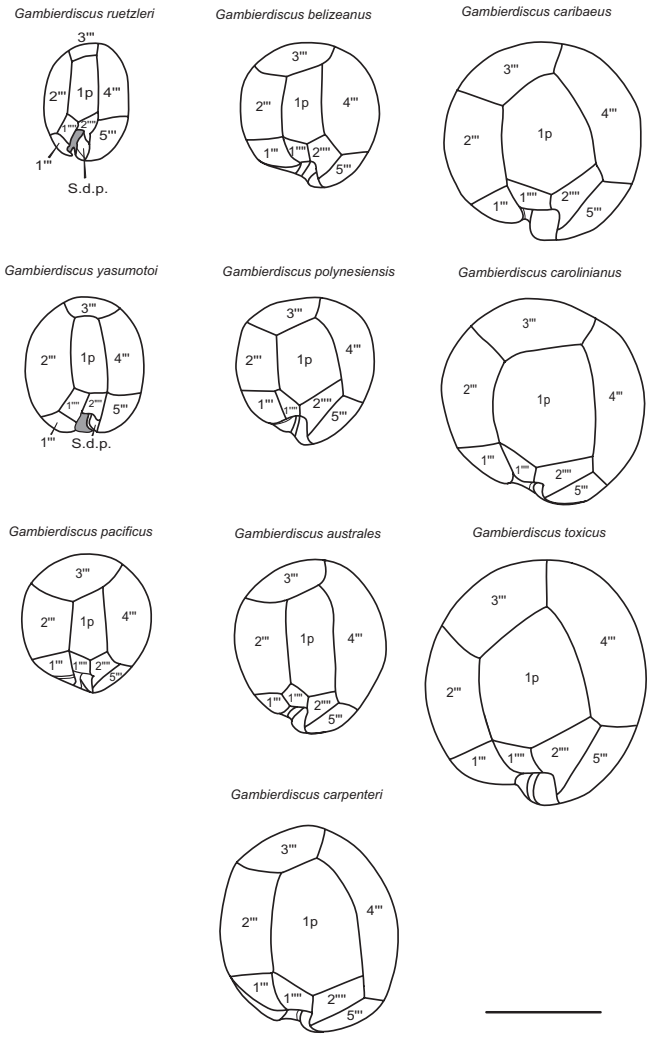


Figure 8. Comparative line drawings of the hypotheca for 10 Gambierdiscus *species. Scale bar = 50 µm. Copyright (2009) International Phycological Society from Phycologia by Litaker et al. (2009). Republished by permission of Allen Press Publishing Services.*

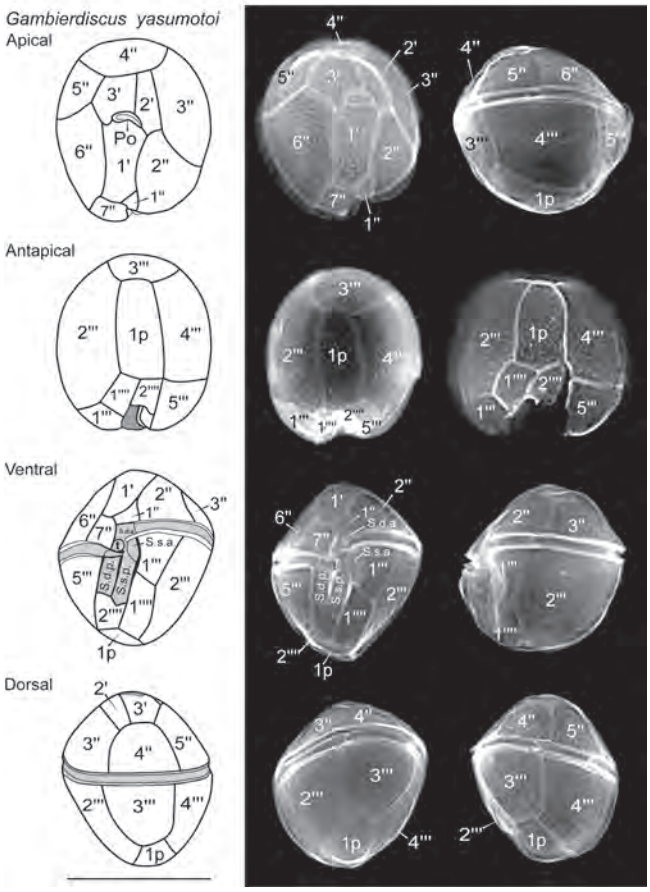


Figure 9. Left, line drawings of the theca for the globular species Gambierdiscus ruetzleri *seen from different perspectives. Right, cells stained with calcofluor, which allows the surface plates covering the cell to be visualized when exposed to ultraviolet light. Scale bar = 50 µm. Right: from Litaker et al. (2009), with permission of Allen Press Publishing Services. For more information on* Gambierdiscus *morphological and molecular taxonomy see: http://gambierdiscuswiki.wikispaces.com/. Semi-quantitative PCR assays are now available for six Caribbean* Gambierdiscus *species to provide assistance with their detection and quantification (Vandersea et al., 2012).*

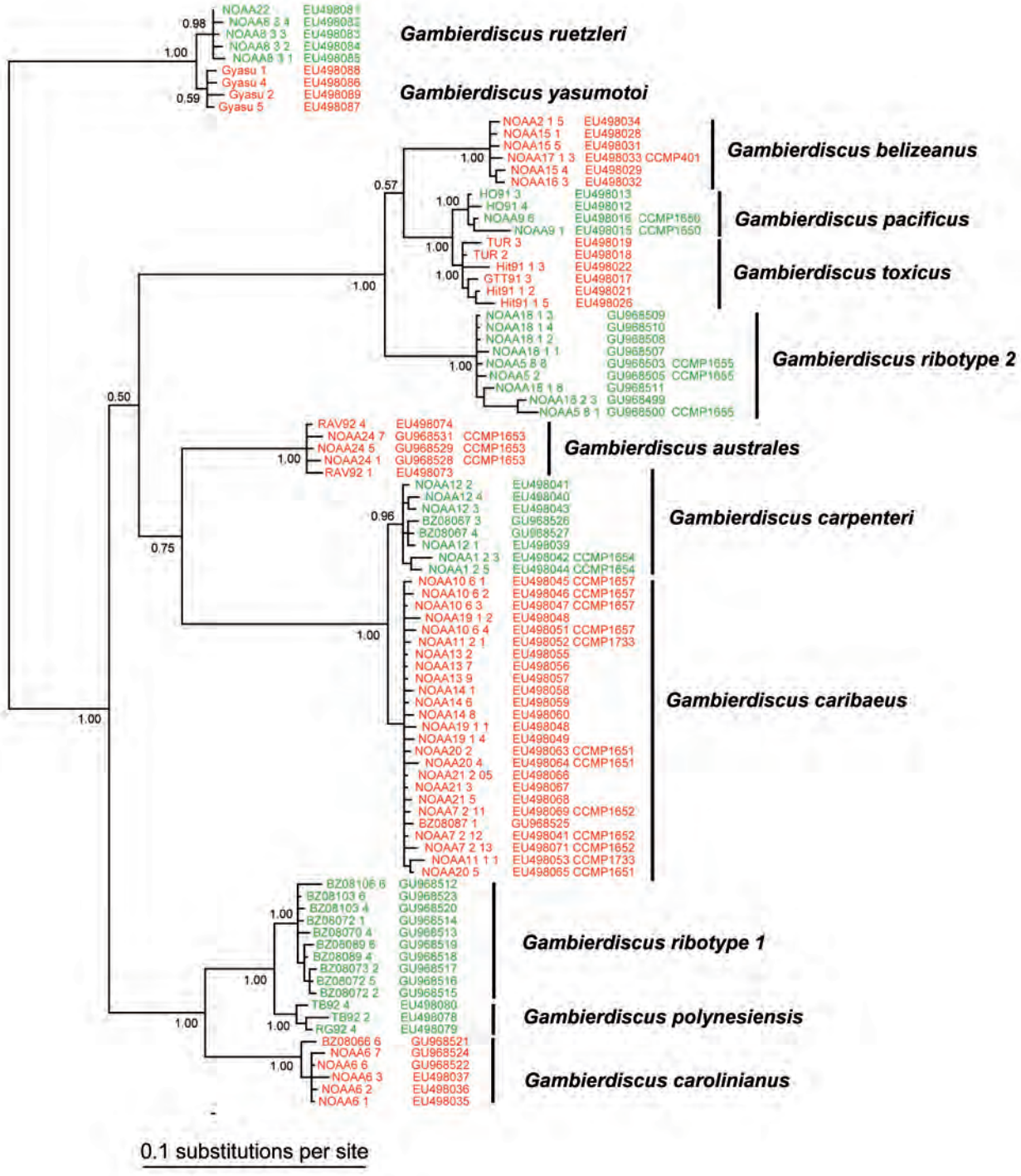


Figure 10. D8-D10 large subunit ribosomal gene phylogeny showing the relationship between the six previously described Gambierdiscus *species and the four newly described species as well as two distinct ribotypes 1 and 2, which may represent new species. Redrawn from Litaker et al. (2009).*

B2. *Ostreopsis*

The genus *Ostreopsis* (Schmidt, 1901) was monospecific for 80 years with *O. siamensis* as the unique species (Figure 11). During that period of time, this species was rarely reported in the scientific literature. However, in a survey to search for the origin of CFP in some Pacific Ocean islands, *Ostreopsis* cells were found and two new species were described, *O. ovata* and *O. lenticularis*, which resulted in a redescription of the type species (Fukuyo, 1981). As the interest in benthic dinoflagellates increased, new species were described including (Table 2): *O. heptagona* (Norris et al., 1985), *O. mascarenensis* (Quod, 1994), *O. labens* (Faust and Morton, 1995), *O. marinus*, *O. belizeanus* and *O. caribbeanus* (Faust, 1999). All these species were described based only on the morphology of cells

from field samples and the importance given to different taxonomic characters depended only on the different author's criteria.

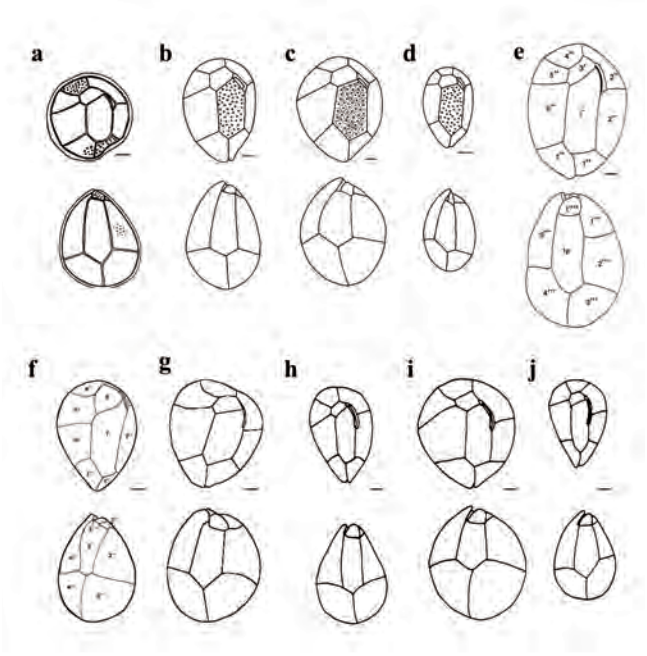


Figure 11. Line drawings of the nine described Ostreopsis *species in epithecal (upper) and hypothecal (lower) view. (a)* O. siamensis *after Schmidt (1901); (b-d)* O. siamensis, O. lenticularis *and* O. ovata, *respectively, after Steidinger and Tangen (1996); (e)* O. mascarenensis, *after Quod (1994); (f)* O. heptagona, *after Norris et al. (1985); (g)* O. labens, *redrawn from Faust and Morton (1985); (h-j)* O. belizeanus, O. marinus, *and* O. caribbeanus, *respectively, after Faust (1999). Scale bars, 10 µm. From Penna et al. (2005), with permission from the editor.*

Most *Ostreopsis* species are markedly anterior-posteriorly compressed, almond-like in shape (Figure 12). So, they are usually observed in apical or antapical view. *O. ovata* is the less compressed and most elongated species in the dorsoventral axis, and has similar transdiameter and anteroposterior dimensions, which confer it an egg-like appearance. Unfortunately, visual discrimination among species is not so simple, as morphological characters (general shape, cell size, plate tabulation, pore sizes) are highly variable (see for instance, Penna et al., 2005). Plate tabulation is the same for all the species except for *O. heptagona* in which plates 4' and 4'' are in contact, while these plates are separated by 3' and 5'' in the other species. Nevertheless, this is not a good morphological character either given its observed variability (Penna et al., 2010). Sato et al. (2011) have also shown that strains attributable to *O.* cf. *ovata* on morphological ground actually belong to distinct genetic lineages.

In fact, an increasing number of genetic studies have shown that the taxonomy of the genus *Ostreopsis* should be redefined. The first *Ostreopsis* genetically sequenced were cultured strains, identified as *O. ovata* and *O. lenticularis*, isolated from Malaysian waters two years after the last of the nine *Ostreopsis* species were described (Pin et al., 2001). Subsequent *Ostreopsis* cf. *ovata* isolates originating from the Mediterranean Sea, NE Atlantic Ocean and from the Brazilian coast were found to be genetically distinct from those from Eastern Asia based on sequence analyses of different ribosomal DNA regions (LSU D1/D2 and ITS-5.8S) (Penna et al., 2005; 2010; Figure 5 in section A2). In these cases, samples were obtained from regions quite distant from the type locality of any of the formally described *Ostreopsis* species. As indicated for *Gambierdiscus*, the morphological differences are not large enough to allow easy identification of the described *Ostreopsis* species. Indeed, it is quite common that the authors express doubts regarding the correct identification. Based on different regions of ribosomal DNA sequences (LSU D8/D10 and ITS-5.8S), Sato et al. (2011) identified eight phylogenetic clades which could correspond to eight different species that are not morphologically characterized yet. Finally, several cryptic species are included in this taxon, which is now to be considered a species-complex.

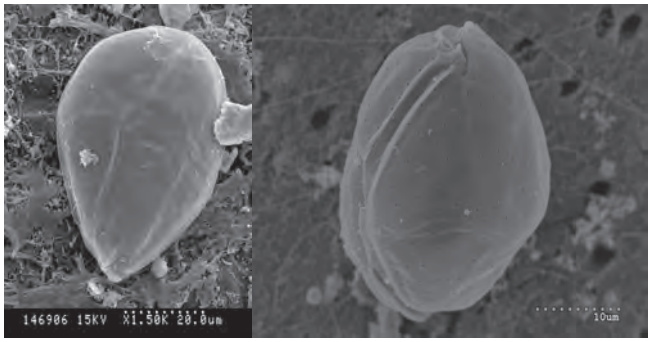


Figure 12. Scanning electron micrographs of Ostreopsis *cf.* siamensis *(left) isolated in the Costa Brava (NW Mediterranean; picture provided by M. Vila and J.M. Fortuño); and* O. *cf.* ovata *(right), IEO-OS15BR from the western Atlantic (from Penna et al., 2005, figure 4, with permission from the editor).*

B3. Characterizing New Species

There is evidence that the diversity of BHAB species is far greater than the number of species described to date. Given that new molecular methods allow rapid sequencing of the rDNA genes from single cells and cultures established from single cell isolates, it is probable that these new species will be first identified genetically. For example, Litaker et al. (2009; 2010) established a one to one correspondence between distinct genetic clusters in rDNA phylogenies and described *Gambierdiscus* species. Also Sato et al. (2011) identified new genetic clusters that do not correspond to the already identified *Ostreopsis* species. Some of them have a high probability of representing new species. To avoid confusion in the literature, a Wiki has been de-

veloped to assist researchers in assigning a sequential "ribotype" number to each new putative *Gambierdiscus* species identified genetically by various research groups around the world (see **http://gambierdiscuswiki.wikispaces.com** for additional details). Because species vary in toxicity (Lartrigue et al., 2009; Chinain et al., 2010b; Litaker et al., 2010; Sato et al., 2011), it will be important to fully characterize *Gambierdiscus* and *Ostreopsis* species diversity in order to understand which species pose the greatest toxicological threat to human and animal populations.

Hence, **a major activity of this CRP will involve both the morphological and genetic characterization of new species**. The molecular characterization will prove particularly important in developing species-specific detection and quantification assays for the toxic species of interest.

C. Biogeography: Distribution and Abundance of BHABs

C1. *Gambierdiscus*

C1.1. Gambierdiscus *Distribution*

Species in the genus *Gambierdiscus* have a pan-tropical distribution between 35°S and 35°N. This genus is reported throughout the Caribbean Sea, the Hawaiian Islands, Australia, Southeast Asia and the Indian Ocean. Recently, *Gambierdiscus* has been found in the Canary Islands, the Mediterranean Sea, the Gulf of Mexico, and in the Atlantic Ocean off the coast of North Carolina, USA (34.7°N) (Villareal et al., 2007; Aligizaki and Nikolaidis, 2008; Litaker et al., 2009; Fraga et al., 2011). *Gambierdiscus* was also observed in the coast of Angola (Isabel Rangel, pers. com.) and ciguatoxins were detected in fish from Cameroon (Bienfang et al., 2008) but the whole coast of Africa remains largely unexplored.

Until 2009, establishing the distribution of various *Gambierdiscus* species was impeded because the taxonomy of the genus was poorly defined. Studies simply reported counts as *Gambierdiscus* "toxicus" or *Gambierdiscus* sp. (Tester et al., 2006; Litaker et al., 2009). A revision of the genus in 2009, using both morphological and genetic methods, made it possible to unambiguously identify *Gambierdiscus* species. *Gambierdiscus excentricus* was described by Fraga et al. (2011) and another new species is being described from the Mediterranean Sea (Aligizaki pers. com.). Reexamination of the distribution of *Gambierdiscus* species has followed (Litaker et al., 2009). Recent studies using genetic methods indicate marked differences in *Gambierdiscus* species distributions between the Atlantic and Pacific-Indian Oceans (Figure 13). Currently, *G. ruetzleri*, *G. excentricus* and *Gambierdiscus* ribotypes 1 and 2 are found exclusively in the Atlantic Ocean. *Gambierdiscus australes*, *G. pacificus*, *G. polynesiensis*, *G. toxicus*, and *G. yasumotoi* are found only in the Pacific. It should be noted that both *Gambierdiscus toxicus* and *G. yasumotoi* have been reported from the Caribbean. The *G. toxicus* reports preceded the recent taxonomic revisions and likely represent misidentifications. Extensive isolations from field samples as well as direct screening of DNA extracted from environmental samples, have yet to confirm the presence of *G. toxicus* in the Carib-

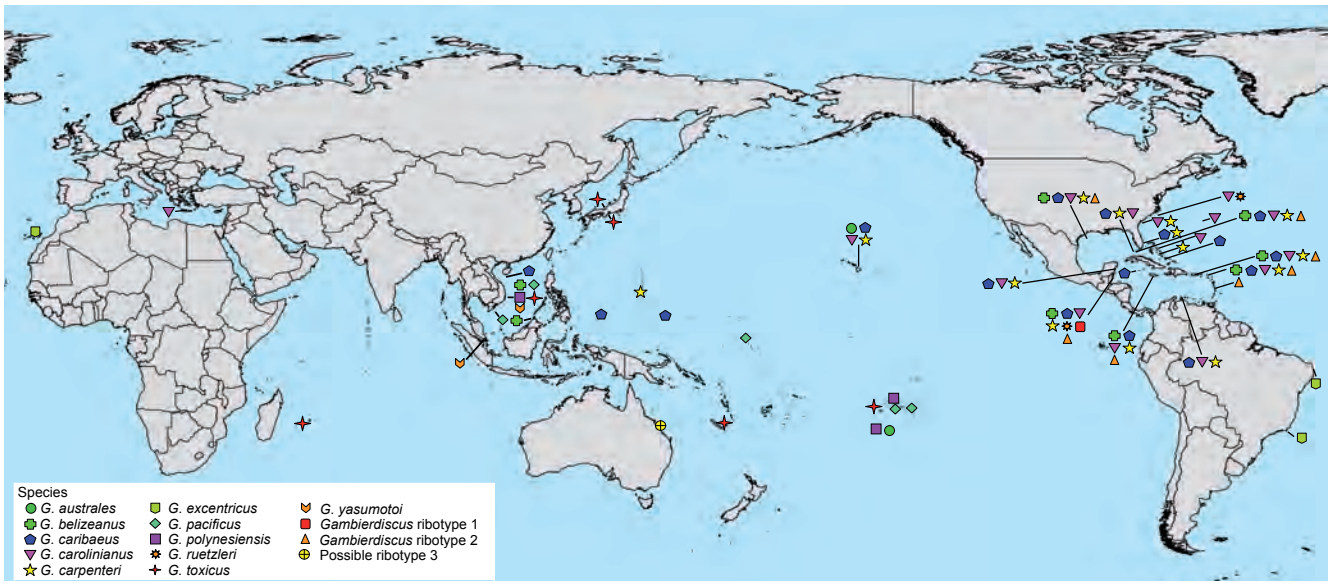


Figure 13. Global distribution of the Gambierdiscus *species listed (Litaker et al. 2010; Ho-Van et al., 2010; Hatayama et al., 2011, Ishikawa et al., 2011; Leau et al., 2011; Nascimento et al., 2012). A new species,* G. excentricus *was isolated from the Canary Island in the northeast Atlantic and described by Fraga et al. (2011).*

bean (Figure 13). Similarly, the report of *G. yasumotoi* were made prior to the description of the closely related species *G. ruetzleri* (Hernández-Becerril and Becerril, 2004). *Gambierdiscus toxicus* is the only species reported from the Indian Ocean and confirmed by molecular methods (Chinain et al., 1999). Four species, *G. belizeanus*, *G. caribaeus*, *G. carolinianus* and *G. carpenteri*, are known to occur in both the Atlantic and Pacific Oceans and are likely globally distributed (Figure 13). *Gambierdiscus caribaeus*, one of the six species tested so far for the effects of temperature on growth, is the most temperature-tolerant species which may partially account for its wide distribution (Kibler et al., 2012).

As discussed in section A3, CFP occurrence, for the most part, supports the expected pantropical distribution of *Gambierdiscus*, but does not fully represent the distribution range of non-toxic species or regions where environmental conditions preclude high enough densities to cause CFP (Litaker et al., 2009; Chinain et al., 2010a). For instance, Caillaud et al. (2010) reported the presence of *Gambierdiscus* in Greece that did not cause any CFP outbreak, so it was not registered in the area.

As with the global distribution of most microorganisms, it is possible with additional samplings, that species now designated as endemic to either the Atlantic or Pacific may eventually be found in both regions. Even if this proves true, it is likely that the relative abundance of certain species will be different between the the Atlantic and Pacific. This conclusion regarding the general pattern of distribution is supported by the fact that, despite screening hundreds of samples at many locations in the Atlantic over the past 5 years, none of the Pacific-specific species has been observed in the tropical or subtropical regions in the Atlantic (Caribbean/Gulf of Mexico/West Indies/Southeast US coast from Florida to North Carolina). Even if the Pacific species are eventually found in the Atlantic, it is expected they will be relatively rare.

C1.2. Gambierdiscus *Abundance*

Good abundance estimates for *Gambierdiscus* species are difficult to find because *Gambierdiscus* cells are hard to both identify and sample. Progress has been made in the identification of *Gambierdiscus* species (Litaker et al., 2009), but quantitative methods for systematic sampling are just now being developed. In addition to taxonomic issues, a primary impediment to progress is that benthic dinoflagellates occupy complex, multidimensional habitats that are very challenging to sample. One method for estimating *Gambierdiscus* abundance commonly used since the late 1970s (Yasumoto et al., 1979; Yasumoto et al., 1980) requires that macroalgae be collected, placed in plastic containers and shaken vigorously to remove attached *Gambierdiscus* cells (see Reguera et al., 2011 and section C3). Note that not all the cells can be detached from their substrate. The suspended cells are then sieved through a mesh screen to remove detritus and larger grazers before *Gambierdiscus* cells are captured on a finer mesh screen. Finally, cells are backwashed off the mesh screen and preserved for counting. At the same time, the macroalgae are blotted dry and weighed. *Gambierdiscus* cell densities are subsequently expressed as cells•g^{-1} wet weight algae. Samples collected this way have consistently shown that *Gambierdiscus* cells are distributed in a very patchy manner, even over small distances (Ballantine et al., 1985; Ballantine et al., 1988; Lobel et al., 1988). Typically, coefficients of variation among adjacent samples ranged from 50 to >150%.

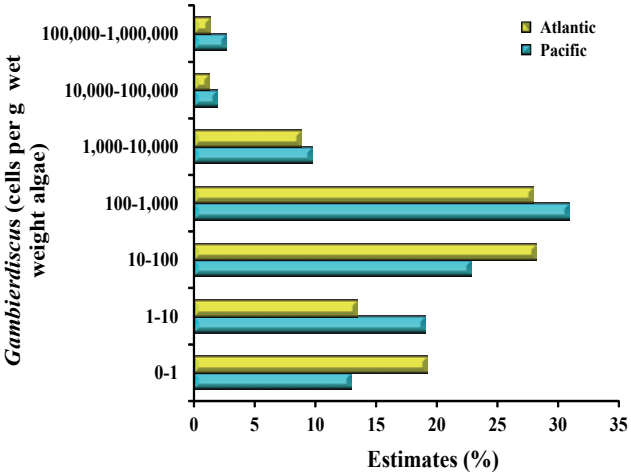


Figure 14. Mean Gambierdiscus *abundances from 46 studies. The 0-1 values are likely underestimated because samples were biased toward sites where* Gambierdiscus *cells were known to be present. N=number of samples analyzed. Redrawn from Litaker et al. (2010).*

Despite this variation, it was possible to collate the available average *Gambierdiscus* abundance data from the literature and estimate representative density distributions for the Atlantic and Pacific oceans (Litaker et al., 2010; Figure 14). The frequency distributions of average cell densities were similar in both the Atlantic and Pacific. Eighty-five percent of the abundance estimates were <1,000 cells g^{-1} wet weight algae. Approximately 10% of the abundance estimates were between 1,000 and 10,000 cells g^{-1} wet weight algae. Estimates exceeding 100,000 cells g^{-1} wet weight algae were fewer than 5% of the total. The only estimate that exceeded 1,000,000 cells g^{-1} wet weight algae was from the Pacific. It is likely that the highest 10% of the densities represent localized epibenthic blooms of *Gambierdiscus* that have been noted in the literature (Nakajima et al., 1981; Withers 1984; Chinain et al., 1999; Darius et al., 2007). It is these bloom populations which are most likely to contribute to CFP events. Sixty-six mean abundance estimates were also found from the Indian Ocean, but represented too small a sample size, both in terms of sample number and the diversity of sites sampled, to estimate average abundances in this region (Grzebyk et al., 1994; Hurbungs et al., 2001; Lugomela, 2006).

C2. *Ostreopsis*

C2.1. Ostreopsis *Distribution*

Benthic dinoflagellates of the genus *Ostreopsis* were first described from the Gulf of Siam (Thailand) at the beginning of the last century (Schmidt, 1902). The type species, *Ostreopsis siamensis*, apparently has a worldwide distribution including the Sea of Japan, East Australia, New Zealand (North Island), Hawaii, México and the Caribbean Sea. It also occurs in the Indian Ocean (Madagascar and Réunion, Mayotte and Rodrigues islands; Rhodes, 2010). *Ostreopsis* cf. *siamensis* has recently been reported in warm temperate Atlantic waters off Morocco and Portugal (Amorin et al., 2010; Bennouna et al., 2010), as well as in the Mediterranean Sea (Aegean Sea, Algerian, French, Italian, Lebanese, Spanish and Tunisian waters; e.g. Illoul et al., 2012, Touharia and Seridji, 2011) and in the Bay of Biscay (Atlantic Spain; Laza-Martinez et al., 2011). However, the identity of this taxon has not been confirmed in these latter reports where the species is reported as *Ostreopsis* cf. *siamensis* (Penna et al., 2010).

Ostreopsis ovata was described about 80 years later as an epiphyte of brown and red macroalgae growing on coral reefs of Ryukyu Islands, French Polynesia and New Caledonia (Fukuyo, 1981). Seven more species were described in the genus in the next 20 years (Faust, 1999), all living in close contact with macroalgae and/or submerged rocks or sediments. These species were reported from tropical and subtropical seas, where they were associated with other epiphytic dinoflagellates such as *Gambierdiscus*, *Coolia* and benthic *Prorocentrum* (e.g., Besada, 1982). In recent years, the range of *Ostreopsis* species has shown an apparent expansion to temperate regions (Shears and Ross, 2009), where cells can form intense blooms (Rhodes, 2010). This is the case for New Zealand (Shears and Ross, 2009), the cold waters of the Japan Sea (Selina, 2010), and the Mediterranean Sea (Vila et al., 2001; Aligizaki and Nikolaidis, 2006; Monti et al., 2007; Mangialajo et al., 2008; Battocchi et al., 2010; Totti et al., 2010; Mangialajo et al., 2011). At the same time, records of *Ostreopsis* have also increased in subtropical-tropical waters, such as Brazil (Rhodes et al., 2009; Nascimento et al., 2011).

The Mediterranean Sea represents the case that best shows the apparent range expansion of *Ostreopsis*. The first record of an *Ostreopsis* bloom in this sea dates back to 1972, when *O. siamensis* (Taylor, 1979) was reported to occur at high concentrations within a mucilaginous matrix covering macroalgae in Villefranche-sur-Mer Bay (NW Mediterranean sea, France; F.J.R. Taylor, pers. comm.). In the light of the recently assessed range of *Ostreopsis* species, it is likely that the attribution to *O. siamensis* was because it was the only species known in the genus at that time, and that in fact, the Villefranche-sur-Mer bloom was also caused by *O.* cf. *ovata.*

In the Mediterranean Sea, *O.* cf. *ovata* was recorded in Lebanese (Abboud-Abi Saab, 1989) and Italian (Tognetto et al., 1995) waters since the 1980s. Simoni et al. (2003) and Sansoni et al. (2003) first described a clear impact on the benthic fauna during a bloom of this species in coastal north Tyrrhenian waters in summer 1998. Molecular investigations have revealed that Mediterranean/Atlantic populations of *O.* cf. *ovata* cluster into the same phylogenetic group, while Pacific isolates are genetically distinct (Penna et al., 2005; 2010; 2012). More recently, four strains from Shikoku in Japanese waters have shown LSU identity with the Mediterranean/Atlantic strains, while three different genetic clades have been found that share the morphology of *O. ovata*, suggesting that it is a species complex involving at least three cryptic species (Sato et al., 2011). Further phylogenetic analyses based on LSU (D1/D2) regions and including worldwide isolated strains evidenced new clades probably correspond to different species within *Ostreopsis* spp. (Penna et al., 2012; Figure 15). In particular one clade, represented by sequences of *Ostreopsis* sp. isolates from Atlantic and Greek waters, is probably a new species, which is also supported by further morphological and toxin profile characterization (Penna, unpublished). In the absence of molecular information on the type material of *O. ovata*, the taxonomic status of this species remains uncertain. It is therefore advisable to use the designation *Ostreopsis* cf. *ovata* when referring to isolates from both the Mediterranean/Atlantic and the Indo-Pacific clades (Penna et al., 2010) and, more generally to the *O. ovata* species-complex for the taxa attributed to this species after its first description.

Given the complex and partially unresolved status of the genus *Ostreopsis*, it becomes extremely important to identify adequate target molecular regions in nuclear and/or organelle genomes for phylogenetic and phylogeographic studies that can really resolve distinct *Ostreopsis* species. Molecular results should be combined with new morphological characters, as well as with data on toxin profiles and physiological traits. Finally, in order to identify and quantify individual species in environmental samples, it would be necessary to identify adequate molecular regions in the *Ostreopsis* genome for the design of species-specific probes and primers (Perini et al. 2011).

Much less information is available on the geographic range of other species in the genus *Ostreopsis*, which seem to be more strictly confined to tropical and subtropical waters. *Ostreopsis heptagona* occurs in the Gulf of Mexico, Caribbean Sea and Indian Ocean (Réunion and Rodrigues islands). *Ostreopsis labens* was found in Belizean and Puerto Rican waters and

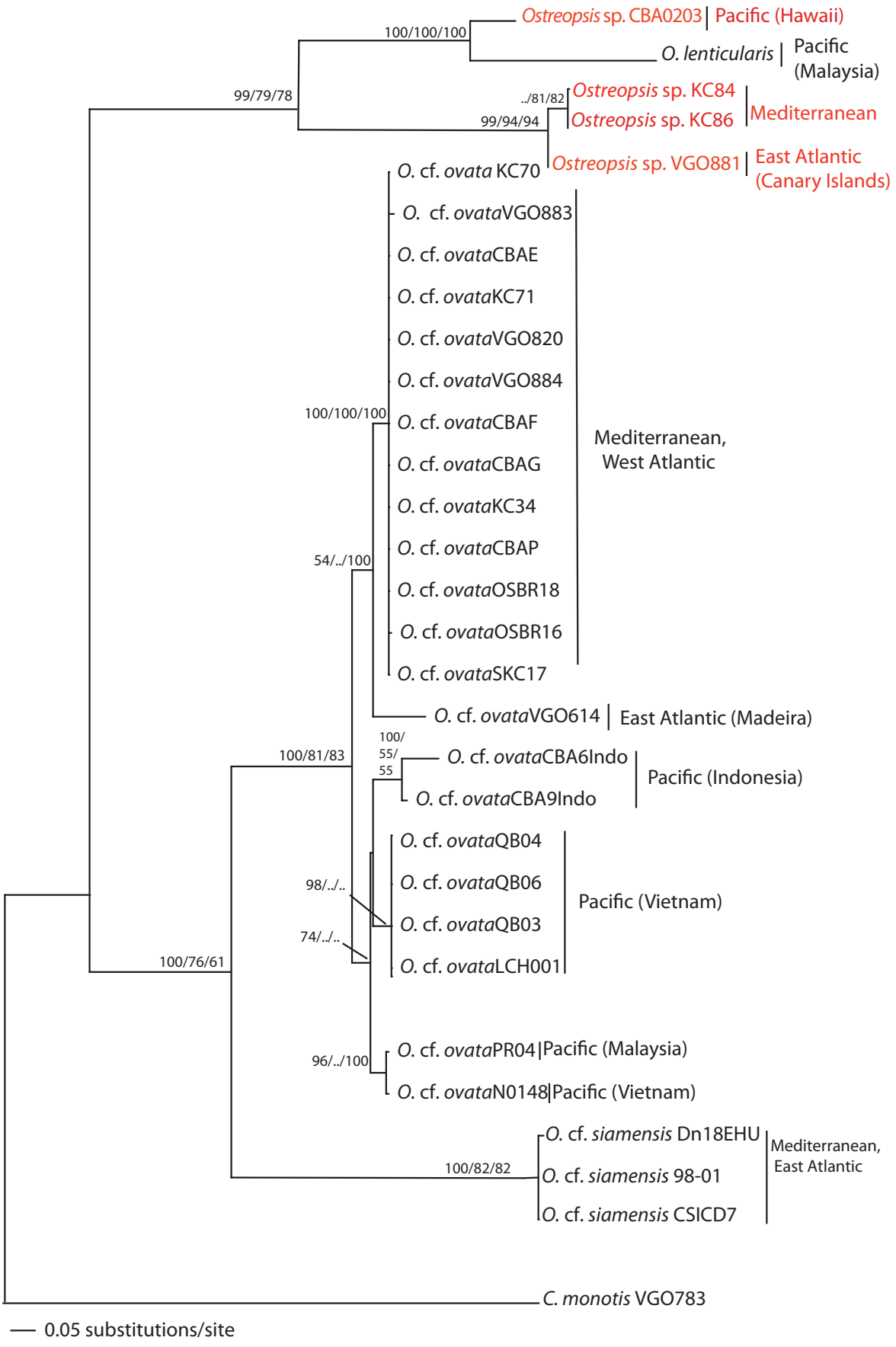


Figure 15. LSU (D1/D2) ribosomal gene phylogeny of genus Ostreopsis *showing relationships among different species including a new Mediterranean/Atlantic clade. Redrawn from Penna et al. (2012).*

Indonesian and Malaysian beaches. The range of *O. lenticularis* range is similar to that of the species mentioned above. *O. marinus*, *O. belizeanus* and *O. caribbeanus* co-occur in the Caribbean Sea and Indian Ocean, whereas *O. mascareniensis* is not present in the northern Australian sea. Nevertheless, the true distribution of the different species is unknown as long as their identity is still doubtful.

C2.2. Ostreopsis *Abundance*

The concentrations reached by *Ostreopsis* species are only known for a few species, while in most cases species are simply recorded as 'present' at given locations or reported as forming a brown film covering the algae but not giving concentrations (Ferreira, 2006).

The exception is represented by *Ostreopsis* cf. *ovata*, which has been intensively sampled around the northern Mediterranean Sea coasts over the last five years. Concentrations at 14 Spanish, French, Monegasque and Italian sites of the north-western Mediterranean coasts reached up to $7.2x10^6$ cells (g wet weight)$^{-1}$ of macroalgae and up to $1.2x10^5$ cells l^{-1}, with a high positive correlation between concentrations recorded in the plankton and in the benthos in the same sampling sites and dates (Figure 16, Mangialajo et al., 2011). Interestingly, peak values were generally recorded in mid-summer (end of July) in the western sites and in early autumn (end of September) in the Adriatic Sea. This difference in seasonal trends is also confirmed from other sites not examined in that study, e.g. the Gulf of Naples (Zingone, unpublished data), where *Ostreopsis* cf. *ovata* peaks in early summer. In Greek waters (Aligizaki and Nikolaidis, 2006), where the peaks are recorded in August-September, and Monaco coast where the peaks are recorded in July and August (Cohu et al., 2011; Mangialajo et al., 2011). A second peak also often occurs in the Catalan coast and in the Gulf of Naples (M. Vila, A. Zingone, pers. comm.). None of the other *Ostreopsis* species has ever been recorded in such high concentrations (Table 3), with the exception of *O. siamensis* found at concentrations near 10^6 cells g^{-1} wet weight in New Zealand waters (Shears and Ross, 2009) and the Costa Brava (Vila et al., 2001).

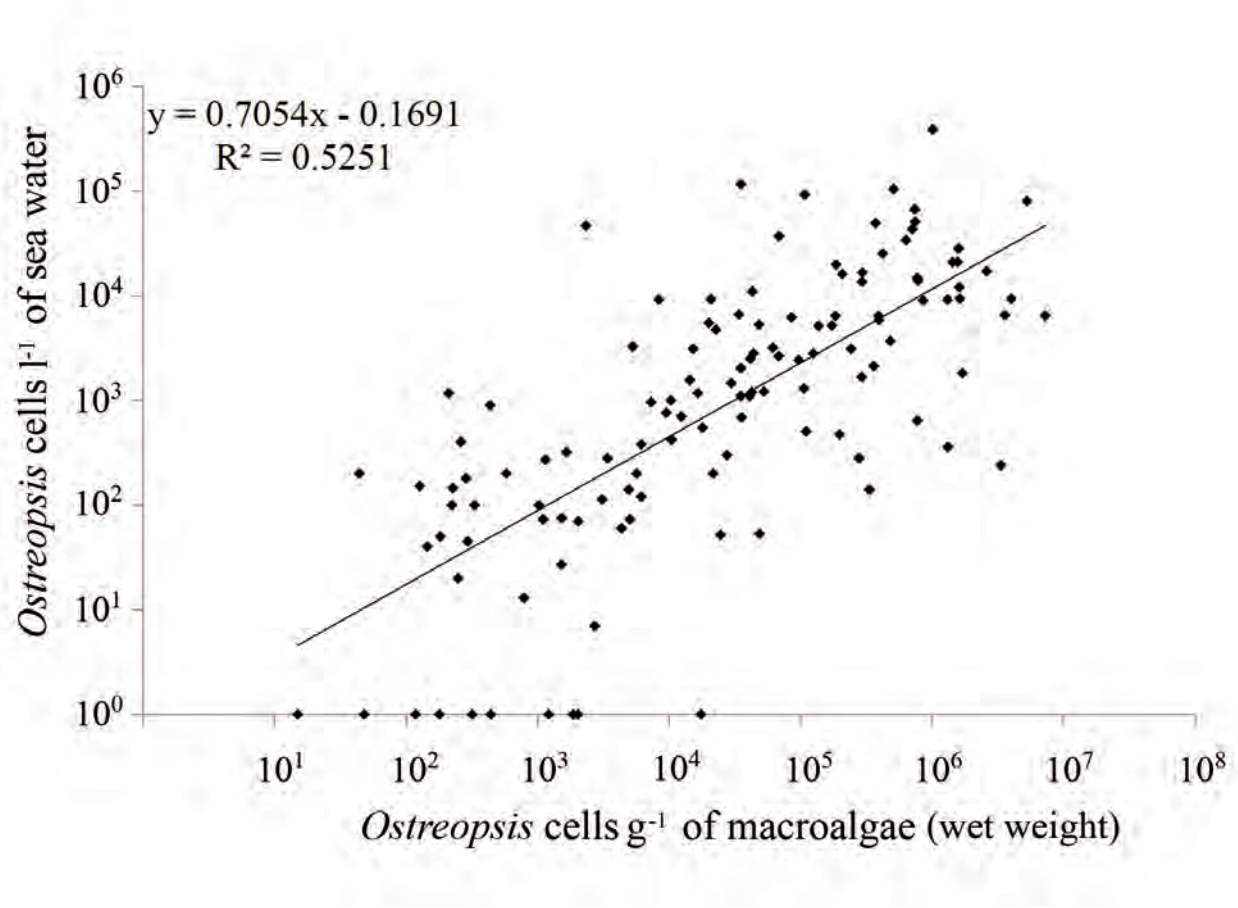


Figure 16. Correlation between maximal concentrations of epiphytic (cells g^{-1} wet weight algae) and planktonic (cells l^{-1}) Ostreopsis *cf.* ovata *at sampling sites from the northern Mediterranean coast (from Mangialajo et al., 2011, with permission from the editor).*

Table 3. Ostreopsis *species abundance estimates.*

Species	Abundance on macrophytes (cells•g^{-1} wet weight)	Abundance in sediment (s) or coral (c) (cells•g^{-1} wet weight)	Water column (cells l^{-1})	Location	Reference
O. cf. *ovata* and *O.* cf. *siamensis*	4.05 × 10^5	135 (s)	1.6 × 10^4	N. Aegean Sea, Greece	Aligizaki and Nikolaidis (2006)
O. lenticularis	1.6 × 10^4			SW Puerto Rico	Ballantine et al. (1988)
O. heptagona *O. siamensis*	<50 <50			SW Puerto Rico	Ballantine et al. (1988)
O. lenticularis	4.3 × 10^4			SW Puerto Rico	Ballantine et al. (1985)
Ostreopsis spp.	55			North Line Islands, Pacific	Briggs and Leff (2007)
O. siamensis	1.12 × 10^5			US Virgin Islands and Caribbean	Carlson (1984)
O. cf. ovata	1.1 × 10^5		≤2 × 10^6 [a]	Monaco, NW Mediterranean	Cohu et al., (2011)
O. lenticularis	<10^3			NW Cuba	Delgado et al. (2006)
Ostreopsis spp.	≤100	≤1900 (c)		Mayotte Island (SW Indian Ocean)	Gryzebyk et al. (1994)
Ostreopsis spp.	≤17			Mauritius (SW Indian Ocean)	Hurbungs et al. (2001)
Ostreopsis spp.	5.7 × 10^4	4.8 × 10^3 (c)		US and British Virgin Islands	Kohler and Kohler (1992)
O. cf. *ovata* *Ostreopsis spp.*	16.4 12.4			Akajima Island, Okinawa, Japan	Koike et al. (1991)
O. siamensis	1-5			Tunisia	Mabrouk et al. (2011)
O. cf. *ovata*	7.2 × 10^6		1.16 × 10^5	NW Mediterranean Sea	Mangialajo et al. (2011)
O. lenticularis *O. heptagona*	1.5 × 10^3 85			Belize	Morton and Faust (1997)
O. cf. *ovata*	7.4 x 10^4		4.0 x 10^4	NW Adriatic, Mediterranean Sea	Perini et al. (2011)
Ostreopsis spp. *O. siamensis* *O. ovata* *O. lenticularis*	≤100 ≤55 ≤60 ≤15			Johnston Atoll, Pacific Ocean	Richelin and Lobel (2011)
O. siamensis	1.4 x 10^6			New Zealand	Shears and Ross (2009)
O. heptagona	1202			Gulf of Mexico	Okolodkov (2007)
O. cf. *siamensis*	5.96 x 10^5			Costa Brava, NW Mediterranean	Vila et al. (2001)[b]

[a] Sample collected in the water directly above targeted macrophytes, [b]Although Vila et al. (2011) refer to *Ostreopsis* spp., a later study (Penna et al., 2005) concluded that the organism was *O.* cf. *siamensis.*

C3. Sampling Methods

BHAB species occupy structurally complex environments. Substrates associated with benthic dinoflagellates include various species of thallic and turf algae, seagrass, sand, pebbles, rocks, coral and coral rubble. This diversity of substrate types greatly complicates the design of quantitative sampling strategies for these organisms. The situation is further complicated by the patchy distribution of benthic HABs, which can rapidly shift even over relatively short time periods. A variety of protocols have been used to sample the heterogeneous environment where BHABs occur, but none has been systematically evaluated. The myriad, non-standardized, sampling methods contrast sharply with pelagic HAB sampling methods, which are well defined and allow meaningful comparisons to be made among different studies. While the need to standardize collection methods for benthic dinoflagellates has long been recognized, the methods for doing so are diverse and challenging. Cell abundance is generally expressed as cells per gram wet weight (or fresh weight) of substrate. Because the substrates are characterized by complex morphologies with a wide range of surface area-to-mass ratios, cell abundances among different substrates are not directly comparable. Reguera et al. (2011) provide detailed sampling methods for both phytoplankton and benthic species.

The existing sampling methods fall into three categories: vacuum collection, substrate sampling and the use of artificial substrates. Each of these approaches has advantages and disadvantages.

C3.1 Vacuum collection

These methods rely primarily on displacing cells from the substrate and capturing those cells by filtration (Parsons et al., 2010; Figure 17). This can usually be accomplished with minimal destruction to the habitat and allows a variety of substrates to be sampled. The number of sampling sites needed to produce an accurate estimate of cell abundance, however, is not known. A major disadvantage of vacuum filtration is that the samples tend to be dirty and hard to count and some substrates, like loose sand or mud, are impossible to sample because they clog the filtration apparatus. The primary advantage of the method is that the sampling can be accomplished in one trip to the sampling site.

C3.2 Substrate Sampling

Macrophyte (substrate) collection and removal have the advantage of being accomplished in a single sampling trip. To date, this has been the primary method used in a majority of studies. Consequently, most of the data reported for benthic cell abundance is normalized to gram wet weight of algae. Despite the wide use of this method, contradictory conclusions can be reached depending on whether the number of dinoflagellates is normalized to algal biomass or surface area (Lobel et al., 1988). In the past, the surface based approach was not practical because no method existed for rapid and accurate measurement of macrophyte surface area. New methods for measuring surface areas, however, have been recently developed to estimate surface areas of the macrophytes found in coral reef ecosystems. These novel approaches (dye, laser) may make it possible to normalize benthic HAB cell densities obtained from substrate sampling to surface area rather than wet or dry weight. A significant disadvantage to macrophyte collection is that numerous samples are required, many of which can be difficult to count due to high concentrations of detritus.

C3.3 Artificial Substrates

A substrate as common as a window screen has the advantage of being relatively easy to deploy in a non-destructive fashion and allows samples to be collected from any location, including the water column (Figure 18). The use of artificial substrates as a potential sampling method is based on the observation that benthic HAB species have been observed to migrate into the water column and colonize new substrates over short distances. This observation leads to the testable

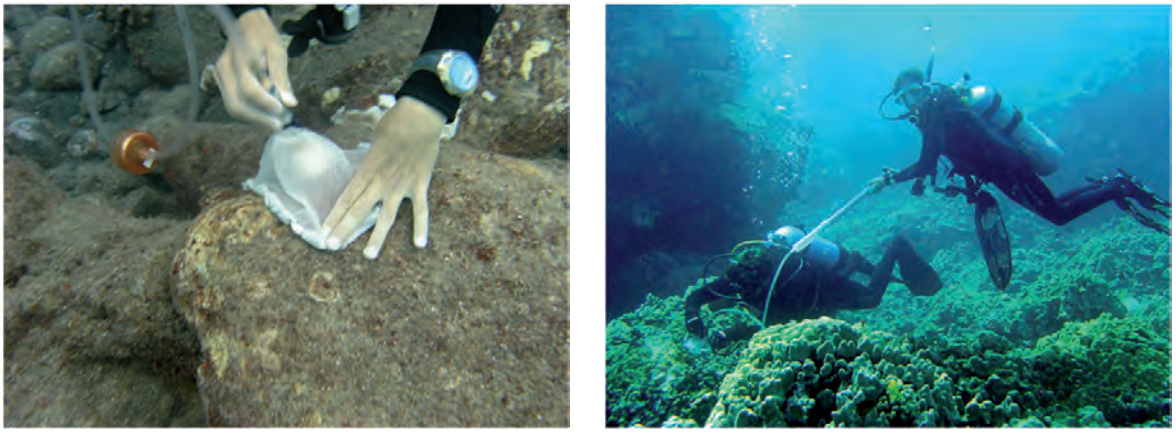


Figure 17. Dino-Vac, the suction-operated sampling device used to collect epiphytes from turf algal substrates (pictures from Parsons et al., 2010, with permission).

hypothesis that benthic cells should recruit to an artificial substrate in proportion to the overall density of the cells in the surrounding habitat. Preliminary data supports such a relationship, but rigorous testing will be required to confirm whether a consistent relationship exists between surrounding cell density and the rate of recruitment to the artificial substrates (Kibler et al., 2008). An advantage of using artificial substrates is that samples are much cleaner than those produced using other methods, which greatly simplifies cell counting. Another advantage of this standardized method is that results can be compared over a variety of different habitats in different geographic regions without dependence on the presence of macrophytes. The major disadvantage is that two trips to the sampling site are required to deploy and then retrieve the artificial substrate.

Figure 18. Artificial substrate. A 750 cm² piece of window screen fabric is used as "artificial seaweed" to collect Gambierdiscus *and* Ostreopsis *cells. This artificial substrate is shown attached to a conch shell with monofilament line in a bed of Acanthophora (NOAA).*

D. Biology of the Organisms

The biology (and ecology) of *Ostreopsis* and *Gambierdiscus* species is difficult to understand due to their complex relationships with substrates and their variable life strategies. These species are commonly classified as benthic or epiphytic algae. However they are often found in the water column and exhibit a different kind of relationship with the substrate as compared to benthic diatoms. Indeed, they are seen swimming freely around the substrate, being only loosely tethered to it, and actively leaving the substrate depending on environmental conditions, e.g. light field (Nakahara et al., 1996). Often these benthic dinoflagellates have been found to produce networks of trichocysts (Barone, 2007 and references there in) and to secrete large amounts of mucilage (Figure 19), forming a dense sheath in which they are embedded (Fukuyo, 1981; Ballantine et al., 1988). This range of conditions (free in the water column, loosely attached to the substrate or embedded in mucilage sheath) could be related to hydrographic conditions and/or physiological status (including life cycle) of these organisms. For example, the presence of certain macroalgae, i.e. *Jania* sp. has been found to promote attachment or swimming behavior, which are also dependent on water motion (Nakahara et al., 1996). The relationships with other environmental parameters (light, nutrients) and potential predators are likely to vary greatly in relation with the diversified life strategies of these species, and in a way that is difficult to reproduce in laboratory experiments. These complex behaviors also contribute to their patchy distribution at local scales. This makes obtaining accurate abundance estimates very difficult. A brief overview of what is known about the effects of nutrients, temperature, salinity, light, water movement and substrate types affecting the ecology and distribution of these species follows.

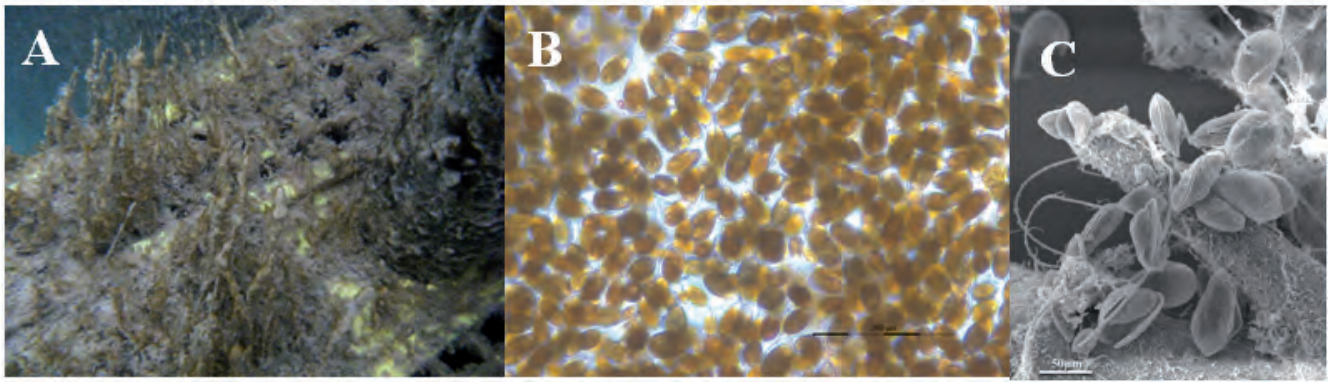


Figure 19. Proliferation of Ostreopsis *cf.* ovata *in Sant Andreu de Llavaneres (NW Mediterranean). (a) Detail of the mucus produced by* Ostreopsis *covering a plastic substrate. (b) Light microscopy picture of* Ostreopsis *cells. (c) Scanning electron images of* Ostreopsis *cells covering a macroalga (*Corallina*) in Palamós (Costa Brava, NW Mediterranean). a and b: images provided by M. Vila (unpublished). c: from Vila et al., 2001, with permission from the editor.*

D1. Nutritional Ecology

D1.1 Gambierdiscus

What we know of the nutritional requirements of *Gambierdiscus* derives almost entirely from culture studies and the results are somewhat contradictory. Early culture studies conducted by Yasumoto et al. (1980) and Withers (1982) revealed that *Gambierdiscus* grew well in the presence of nitrate and either glycerophosphate or inorganic phosphate. Bomber and Aikman (1989) found good growth of *Gambierdiscus* using either nitrate or ammonium as the nitrogen source. Durand-Clement (1987) reported a weak temporary increase in the division rate of *G. toxicus* upon addition of high concentrations of ammonium ($\leq$ 500 µmol l^{-1}), but that the cultures became nitrogen limited after only a few days. Durand-Clement (1987) also noted growth stimulation when urea was added, but there was no effect with amino acid enrichment. Lartigue et al. (2009) studied the growth response of *G. caribaeus* CCMP1651 and *Gambierdiscus* ribotype 2 CCMP1655 to different N-sources and found that both species were able to take advantage of organic (free amino acids and putrescine) as well as inorganic (ammonium and nitrate) nitrogen sources to support growth. Neither species was able to grow on urea, which was contrary to the finding of Durand-Clement (1987). *Gambierdiscus* ribotype 2 grew significantly faster on ammonium than on other nitrogen sources whereas N-source had no effect on the growth rate of *G. caribaeus*. These data suggest that the dominant nitrogen source available to the cells may differentially favor the growth of one *Gambierdiscus* species relative to another.

Lartigue et al. (2009) found that the nitrogen source used to grow two different *Gambierdiscus* species had no apparent effect on toxicity. In contrast, the N:P ratio does seem to affect toxicity. Sperr and Doucette (1996) found that some *Gambierdiscus* strains which were nontoxic could be stimulated to produce ciguatoxins under P-limitation (N:P>30). However, the amount of induction was modest. Beyond the work of Sperr and Doucette (1996) nothing is known about how phosphate limitation affects the growth of *Gambierdiscus* species. This is of interest, because many tropical environments may be phosphate rather than nitrogen limited.

Besides macronutrients, other culture additives such as soil extract in Erdschreiber medium are known to stimulate *Gambierdiscus* growth. Macroalgal extracts added to culture medium all resulted in enhanced growth (Withers et al., 1981; Carlson et al., 1984 and Ballantine et al., 1992). Bomber et al. (1988) similarly used a macroalgal extract to obtain very high growth rates (0.58 divisions day^{-1}). They hypothesized that the extract acted as a complexing agent for trace metals, lessening toxicity of zinc and other metals. In contrast, Parsons et al. (2011) suggested that compounds released by macroalgae may have either an inhibitory or a stimulatory effect on *Gambierdiscus* cells, depending on the macroalgal species. These results seem to suggest that exudates from macrophytes generally stimulate growth of *Gambierdiscus* species but this is not always the case (Carlson et al., 1984; Parsons et al., 2011).

Typical of many dinoflagellates, *Gambierdiscus* species appear to be adaptable, maintaining growth using different organic and inorganic nitrogen compounds. While this flexibility undoubtedly accounts for the wide distribution of many species, more exacting experiments and improved sampling methods will be required to determine the roles of nutrient inputs and macroalgal exudates on nutritional ecology of individual *Gambierdiscus* species. The possibility that ***Gambierdiscus* could have a mixotrophic behavior** under certain circumstances as it is known in other dinoflagellates can not be discarded either.

Another relevant issue is whether nutrient availability can affect toxicity. A recent survey indicated that differences in nutrient availability may cause between a 2 to 9 fold change in the toxicity of *Gambierdiscus* species (Litaker et al., 2010). The relevant data upon which this range was estimated, however, were extremely limited. **The full extent of how nutrient supply may affect both the growth of individual species and their toxicity will be a major component of the CRP.**

Finally, while *Gambierdiscus* is not an obligate epiphyte, the nutritional advantage of its association with macroalgae or surface films on abiotic substrates is not well understood. Tosteson et al., (1989) examined the effect of associated bacteria on toxicity of *Gambierdiscus* cultures and that bacteria may have an effect on both the growth and the toxicity of *Gambierdiscus* cells. Durand-Clement (1987) noted increased *Gambierdiscus* growth rates when associated bacterial populations were controlled with antibiotics. **This topic, because of its inherent importance to both the nutritional ecology and trophic transfer of toxins, is expected to be a very active area of research during this CRP.**

D1.2 Ostreopsis

High nutrient loads and eutrophication have been associated in diverse conditions with the development and apparent expansion of planktonic HABs (e.g. Smayda, 1989; GEOHAB, 2006). However, the diversity of nutrient sources in HABs and the wide gaps in knowledge often make the linkage elusive (Zingone and Wyatt, 2005; Anderson et al., 2008; Smayda, 2008). In the case of *Ostreopsis* and other benthic dinoflagellate blooms, data are very scant and the evidence for a direct or indirect relationship with nutrients is uncertain. The few available data suggest that the distribution of *Ostreopsis* blooms in coastal Mediterranean waters does not mirror that of the most eutrophied zones in this area (e.g. Vila et al., 2001; Cohu et al., 2011; Mangialajo

et al., 2011). Along Hawaiian coasts, the density of *Ostreopsis* sp. was positively correlated with concentrations of several nutrients, but *O. ovata* exhibited no correlation with nutrient availability (Parson and Preskitt, 2007). In the Campania region (Tyrrhenian Sea, Mediterranean Sea), the highest concentrations of *Ostreopsis* cf. *ovata* are found in areas with relatively low nutrient concentrations (Zingone, unpublished data), while *Ostreopsis* and other benthic dinoflagellates are absent or rare in the most eutrophied areas. Nutrients, however, could have an indirect effect on benthic dinoflagellates by favoring, along with reduced grazing impact, the growth of the macroalgae that provide optimal substrate for epiphytic species (Lapointe et al., 2010).

The lack of a clear relationship with nutrients in the natural environment could be because high nutrient inputs stimulate diatom blooms in both the water column and in the benthos, thus resulting in competition for both light and substrate among benthic dinoflagellates (Aligizaki and Nikolaidis, 2006). Among nutrients, phosphorus, and especially its organic forms could be the limiting element (e.g., Cohu et al., 2011). Field studies, usually based on the estimation of inorganic forms, should incorporate measurements of organic P and N as well.

The ingestion of small prey could also be a way to escape nutrient limitation. Indeed phagotrophy seems to be quite widespread among *Ostreopsis* species as in most dinoflagellates (e.g., Stoecker, 1999; Jeong et al., 2010). In a study on natural communities from Belize, *Ostreopsis* cells containing prey were found to constitute 7–55% of the whole natural populations (Faust, 1998). These organisms would feed on flagellates and ciliates through a feeding canal located in the cingulum, which expands or contracts to allow for the engulfment of the prey (Faust, 1998).

By now, only a few studies have dealt with the variability of *Ostreopsis* cell toxicity. Some works showed that toxin production can be higher during the stationary phase of cell growth (Guerrini et al., 2010; Nascimento et al., 2012), when nutrient deficiency is common. However, Vanucci et al. (2012) have shown that toxin production in late stationary phase was significantly lower under N- and P-limitation, with a reduction of 53% and 40% in toxin cell content, respectively. Toxicity also varied with temperature (Vidyarathna and Granéli, 2012), although this could be an indirect effect, given the variability of growth responses as a function of this factor.

D2. Effects of Physical Factors

D2.1 Gambierdiscus

Numerous studies have examined the effects of temperature, salinity and light (Taylor, 1985; Indelicato and Watson, 1986; Taylor and Gustavson, 1986; Bomber et al., 1988; Villareal and Morton, 2002) on the growth rate and biomass yield of *Gambierdiscus* in culture, and on its natural distribution.

D2.1.1 Temperature

In general, the studies on the effects of temperature on *Gambierdiscus* (e.g. Carlson and Tindall 1985; Gillespie et al., 1985; Bomber et al., 1988; Morton et al., 1992; Heil et al., 1998) indicate that its optimal growth occurs generally between 25-29°C. As temperatures exceeded 30°C *Gambierdiscus* growth or abundance was negatively correlated with water temperatures. While each of these studies provides insight on the responses of the genus to this environmental factor, species-specific responses were not possible until

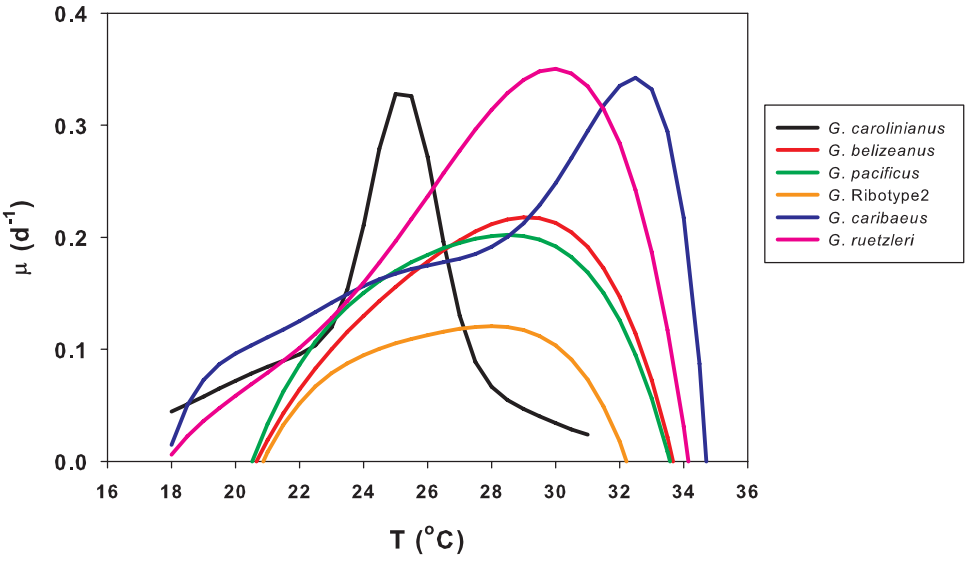


Figure 20. Growth rate (μ, d^{-1}) versus temperature (T (°C)) for six species of Gambierdiscus *at temperatures from 15 to 34°C. Redrawn from Kibler et al. (2012).*

the recent revision of the taxonomy for this group. A study by Kibler et al. (2012) indicates that the growth temperature relationship for individual species can be quite different (Figure 20). Temperature therefore likely plays a significant role in determining the distribution of individual species.

D2.1.2 Salinity

The laboratory studies evaluating salinity range tolerance by *Gambierdiscus* indicate that this organism grows best between 25 and 35 with optima of 28 and 34 (e.g. Bomber et al., 1988; Morton et al., 1992). The data for eight *Gambierdiscus* species shown in Figure 21 (redrawn from Kibler et al., 2012) corroborate this, with optimal growth generally occurring between 25 and 35, but declining rapidly as salinitiy drops below 25. This lack of tolerance to lower salinity is consistent with the results of several field surveys in Caribbean areas (Carlson and Tindall, 1985; Taylor, 1985; Tindall and Morton, 1998) and the SW Indian Ocean (Grzebyk et al., 1994), which suggest that these organisms have a poor tolerance for land runoff where salinity values are sufficiently low. The decline in growth rate at salinity >35 was substantial, but not as dramatic as observed when values declined below 20 (Figure 21). The broad optimum for growth when salinity exceeds 25 indicates that this factor will influence growth, but to a lesser extent than temperature. Where the effects of salinity on growth are likely to be most pronounced, and where salinities may have the greatest influence, in selecting for one species over another, is when prevailing salinity values are <20 and > 35 (Figure 21).

D2.1.3 Light

The ubiquity of *Gambierdiscus* at shallow depths of 0.5 – 50 m (but exceptionally much deeper, at 208 m; Taylor and Gustavson, 1986) means that cells must contend with potentially damaging levels of PAR and UV radiation (Taylor, 1985; Ballantine, 1992; Bomber et al., 1988; Morton et al., 1992). Despite living in high light environments (Figure 22; Vonder et al., 1969; Falkowski et al., 1990), work by Bomber et al. (1988) and Morton et al. (1992) indicate that *Gambierdiscus* only required 230-250 µmol photons $m^{-2}s^{-1}$, or only 10% of the ambient surface irradiance to maintain maximal growth (Guillard and Keller, 1984). Ballantine et al. (1992) demonstrated that optimal growth of the isolate they tested needed only 35 µmol photons $m^{-2}s^{-1}$ for maximal growth. The data from eight *Gambierdiscus* species from Kibler et al. (2012; Figure 23) showed that *Gambierdiscus* species were indeed able to maintain positive growth rates at only 6-17 µmol photons m^{-2} s^{-1} and that maximal growth required only 50 and 230 µmol photons m^{-2} s^{-1}, or ~2.5 to 10% surface irradiance. These data indicate that that *Gambierdiscus* species must utilize physiological as well as behavioral mechanisms, such as using 3-dimensional structures as sunscreens, to deal with high irradiances, which are clearly detrimental to growth (Figure 23). Given the relatively clear waters found in many tropical regions, these data would indicate that actively growing *Gambierdiscus* cells may exist at depths of 75 m or more, provided there is enough light for photosynthesis. There is a great need for additional surveys from deeper water habitats to establish how deeply *Gambierdiscus* species are distributed and the extent to which they

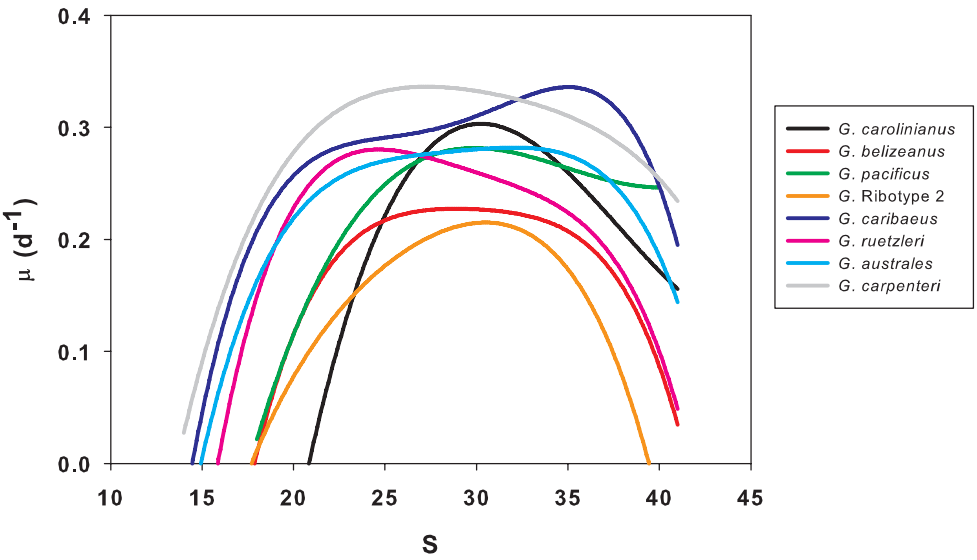


Figure 21. Growth rate (µ, d^{-1}) versus salinity (S) for eigth species of Gambierdiscus *at salinities from 15 to 42. Redrawn from Kibler et al. (2012).*

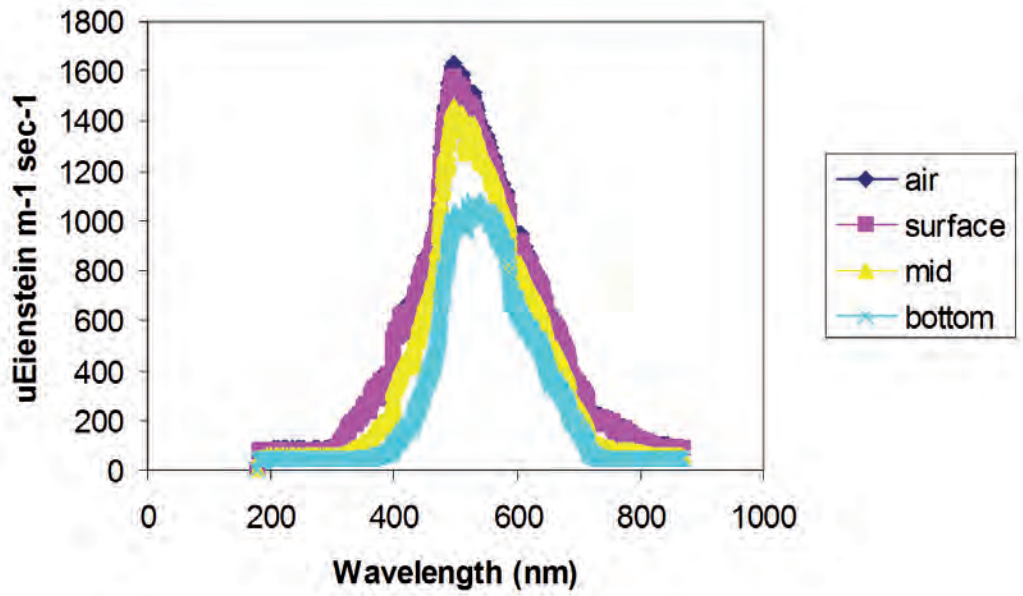


Figure 22. Typical light levels for shallow, tropical waters. Douglas Cay, Belize 16° 46'N, 87° 54'W (P.A. Tester, unpublished data).

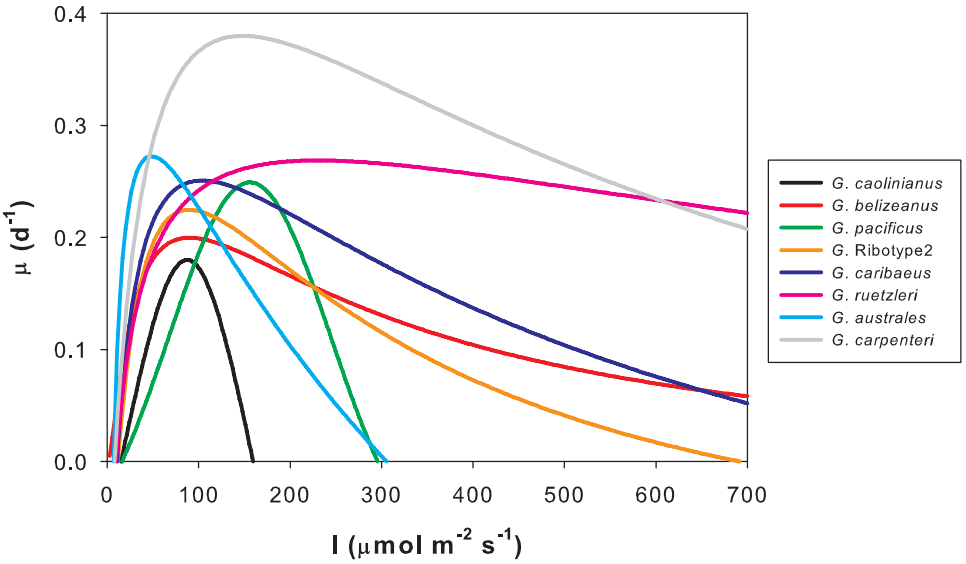


Figure 23. Growth rate (μ, d^{-1}) of eight Gambierdiscus *species at irradiance (I, μmol photons $m^{-2}s^{-1}$) levels between 10 and 700 μmol $m^{-2}s^{-1}$. Redrawn from Kibler et al. (2012).*

may be contributing to the development of ciguatera fish poisoning events in deep water habitats.

D2.2 *Ostreopsis*

D2.2.1 Temperature

First records of *Ostreopsis* in subtropical and tropical waters suggested that the species grows better in warm waters, which was further supported by the prevailing occurrence of the species in summer in temperate waters. However, the few species in the genus that have been tested for optimum growth conditions and tolerance limits to temperature show adaptation to warm temperate rather than to truly subtropical-tropical conditions. For example, in comparison to a number of other species associated with ciguatera (*O. siamensis* and *O. heptagona*) were apparently adapted to a lower optimum temperature of 25 °C. Both species showed a limited tolerance to temperature higher than 27°C. Indeed, *Ostreopsis* species in subtropical waters shows a tendency to have higher densities during the cooler winter months (Morton et al., 1992). Along the Tasmanian coasts, *Ostreopsis siamensis* was found at temperatures between 13.5 and 20 °C, while strains grew at 0.53 divisions•day^{-1} at 20°C (Pearce et al., 2001).

Optimal growth corresponds to moderately high temperatures for *Ostreopsis ovata* from Brazilian waters, where growth rates did not differ significantly between 20°C and 26°C (Nascimento and Corrêa, 2010). Adaptation to temperature may vary among popula-

tions within the same species. In strains from the Mediterranean Sea, the highest growth rates were recorded at temperature values of 22°C and 26°C, with a peak of 1.2 divisions•day^{-1} at 26°C (Zingone et al., 2010; Scalco et al., 2012), whereas another Mediterranean strain grew better and with a maximum cell yields at 30°C as compared to 26°C (Granéli et al., 2010). From field studies, the relationship with temperature is even less clear (Cohu et al., 2011; Mangialajo et al., 2011; Pistocchi et al., 2011). For example, in the Adriatic Sea, *Ostreopsis* blooms occur at the end of summer-early autumn, when seawater temperature values are decreasing and can be as low as ca. 17°C (Totti et al., 2010). This could again be due to differences among populations from different areas, although strains from the Adriatic Sea did not consistently show a preference for low temperature values as compared to Tyrrhenian strains (Scalco et al., 2012).

D2.2.2 Salinity

While the data are scarce the relationship between *Ostreopsis* and salinity does not seem to be linear. Apparently these species can grow and thrive in waters with high salinity. For example, in the Mediterranean Sea blooms of *Ostreopsis* species occur in waters with rather high (<37) salinity values, while benthic dinoflagellates are found in the Florida Keys where salinity may exceed 40 (Morton et al., 1992). However, a negative correlation with salinity was found for *O.* cf. *ovata* along Hawaiian coast (Parsons and Preskitt, 2007), with the highest abundances in places with average salinity values around 26. In the same study, another undescribed *Ostreopsis* species (*Ostreopsis* sp. 1) showed no significant relationship with salinity, and a similar absence of relationship was described for *O. heptagona* in the Gulf of Mexico (Okolodkov et al., 2007). In laboratory experiments, *O. heptagona* and *O.* cf. *siamensis*, along with other co-occurring benthic dinoflagellates, showed optimal growth at a salinity of 33 (Morton et al., 1992). In any case, it is commonly observed that these species are absent in waters under the direct influence of rivers, where salinity can be very low (Pistocchi et al., 2011).

D2.2.3 Light

Like other benthic dinoflagellates *Ostreopsis* species are better acclimated to relatively low light intensity (Rodríguez et al., 2010), developing fast photoprotective responses (increases in non-photochemical quenching (NPQ) at low light intensities) and lower relative electron transport rates (rETR) in comparison with planktonic species. *Ostreopsis* cf. *siamensis* and *O. heptagona* showed optimal growth at ca 8% of full sunlight in one of the few experimental studies on these species (Morton et al., 1992), where the relevance of the interplay of light, temperature and salinity was

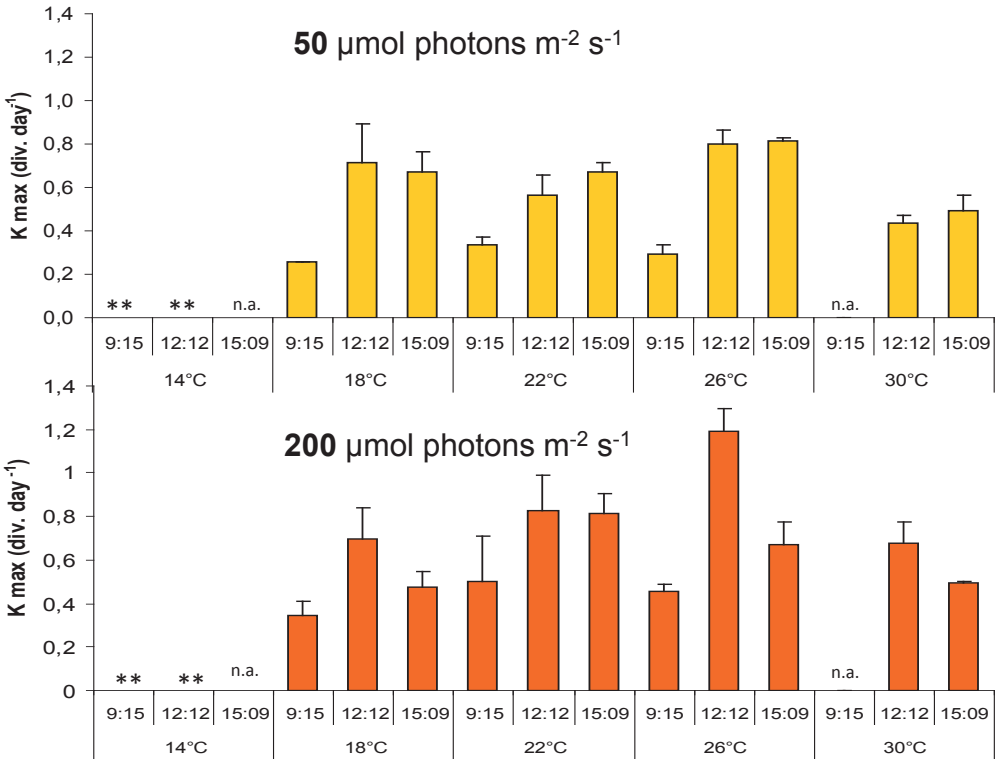


Figure 24. Average maximum specific growth rate (div•day^{-1}) (± s.d.; n = 6) of Ostreopsis *cf.* ovata *strains at the different temperature, daylength (9, 12 or 15 light hours, indicated by 9L, 12L, 15L, respectively), and photon flux density (yellow bars: 50 µmol photons m^{-2} s^{-1}, orange bars: 200 µmol photons m^{-2} s^{-1}) conditions. **Conditions at which maximum specific growth rate was extremely low, not allowing a correct estimate; n.a. = data not available (redrawn from Scalco et al., 2012).*

also shown. Indeed the relationships with light for *O.* cf. *ovata* from the Mediterranean Sea is complex and nonlinear, with growth depending on photoperiod more than on light intensity and appears to be saturated at relatively low total irradiance values (Scalco et al., 2012; Figure 24).

D3. Substrate Preference and Effects of Water Motion

D3.1 Substrate Preference

Gambierdiscus and *Ostreopsis* species have been primarily found on macrophytes (e.g. Vila et al., 2001). However, in several cases they have been reported to colonize organic and inorganic substrates such as biodetritus pebbles, rocks and sand (e.g. Totti et al., 2010). Cells can also proliferate in a mucous matrix in a mucous matrix or dead corals (Gillespi et al., 1985; Grzebyk et al., 1994). The relationship of *Gambierdiscus* and *Ostreopsis* species with specific macroalgal substrates is not clear. In two studies conducted around the Big Island (Hawaii) and in the NW Mediterranean (Monaco, Villefranche-sur-mer), the preference for specific macroalgae varied among species of benthic dinoflagellates, indicating that host specificity was not simply due to different shape of the macroalgae, but was possibly due to particular requirements of the epiphytes, which could be related either to ecological conditions at different sites or to species-specific host-epiphyte interactions (Parsons and Preskitt, 2007; Cohu and Lemée, in press). However, the seasonal patterns of the benthic dinoflagellate assemblages may be linked to the same factors that modulate the dynamics of the macroalgae, namely temperature, irradiance, hydrodynamic regime and availability of substrate (Vila et al., 2001; Tester et al., 2009).

The capacity of the benthic dinoflagellates to produce large quantities of mucilage (Figure 19A), would allow them to attach to the diverse surfaces and to prevent cell dispersion when exposed to strong water motion. Microscopic observation of *G. toxicus* revealed that cells living on the macroalgal surface were covered by a mucous layer or aggregated within a mucilaginous matrix (Yasumoto et al., 1980; Fukuyo 1981; Besada et al., 1982; Ballantine et al., 1988). Nakahara et al. (1996) found that *G. toxicus* cells attached themselves to macroalgae thalli using a short thread; notably, disturbance appeared to stimulate substrate attachment.

D3.2 Water Motion

In general, and within a particular habitat, the highest cell abundances of benthic dinoflagellates (*Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, *Amphidinium*) are recorded under relatively stable water column conditions where losses due to currents and turbulent dispersion are low (e.g. Carlson and Tindall, 1985; Taylor, 1985; Tindall and Morton, 1998; Vila et al., 2001; Richlen and Lobel, 2011). Reduced dispersion promotes colonization of substrates, but has the disadvantage of also lowering gas exchange, nutrient supply and even light exposure. Water motion should therefore be inversely related to the carrying capacity of a macroalga to sustain a benthic dinoflagellate community (Lobel et al., 1988; Tindall and Morton, 1998). It would also interfere with its 3-dimensional organization of the community and may directly affect

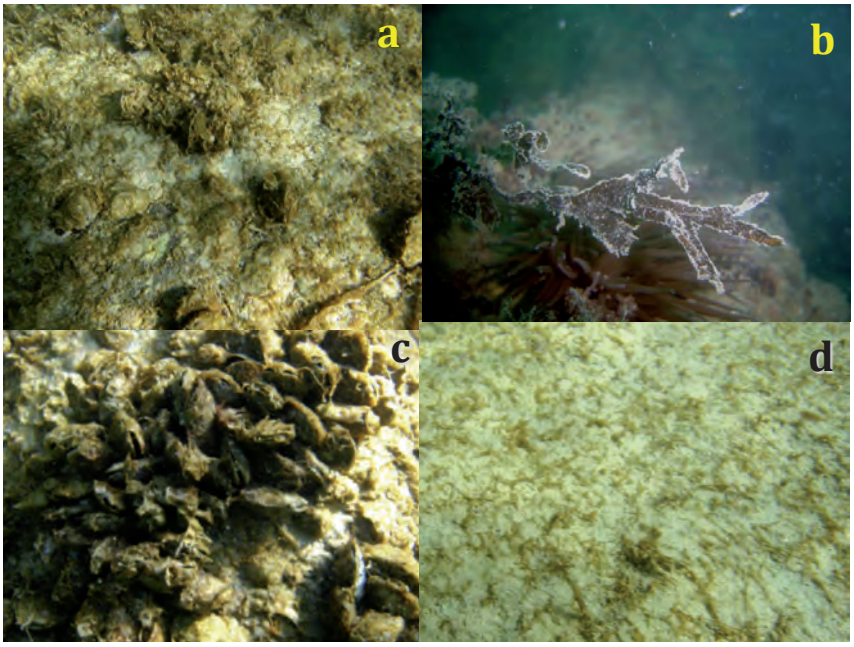


Figure 25. Ostreopsis *cf.* ovata *may produce mucous substances that favour its attachment to several types of substrate. Brownish mucous aggregates containing high numbers of* O. *cf.* ovata *cells can be found, in addition to macroalgae, on rocks (a), marine phanerogams (b), benthic animals (c) and even on rocks (d) (courtesy of C. Totti and M. Vila, unpublished).*

the light environment experienced by *Gambierdiscus* by increasing turbidity, changing the motion of the macrophyte hosts, or by dispersing cells into the water column. Thus the interaction between water motion, substrate characteristics and light exposures may relate in a complex manner that is difficult to separate (e.g. Grzebyk et al., 1994).

Given the lack of quantitative characterization of water motion in most studies, direct comparison cannot be done. There are no standard methods currently in use for estimating water motion in the very shallow environments where benthic dinoflagellate assemblages occur. In a recent study, Richlen and Lobel (2011) used "clod-cards technique" to provide a dimensionless diffusion index factor (DF) as a proxy for water motion (Doty, 1971, revised by Jokiel and Morrissey, 1993). The DF is calculated by dividing the weight loss experienced by the control clod cards into the weight loss experienced by cards deployed in the field. *Gambierdiscus* cells were found at all sampling stations and in all habitats, including the shallow reef crest sites exposed to the brunt of wave action, though they were rarely the dominants. When all the data were collated, they clearly showed a significant negative correlation between water motion and *Gambierdiscus* abundance.

Similarly, the abundances of *Ostreopsis* can be modulated, in general, by water motion. However, some studies have reported that *Ostreopsis* spp., compared to other taxa, seems to tolerate habitats exposed to relatively high water motion. For instance, Vila et al. (2001) reported that in April 1998 in a particular station of the NW Mediterranean coast, *Ostreopsis* cf. *siamensis* (epiphyte of *Corallina elongata*) was more abundant in "high hydrodynamic regimes", defined *sensu*

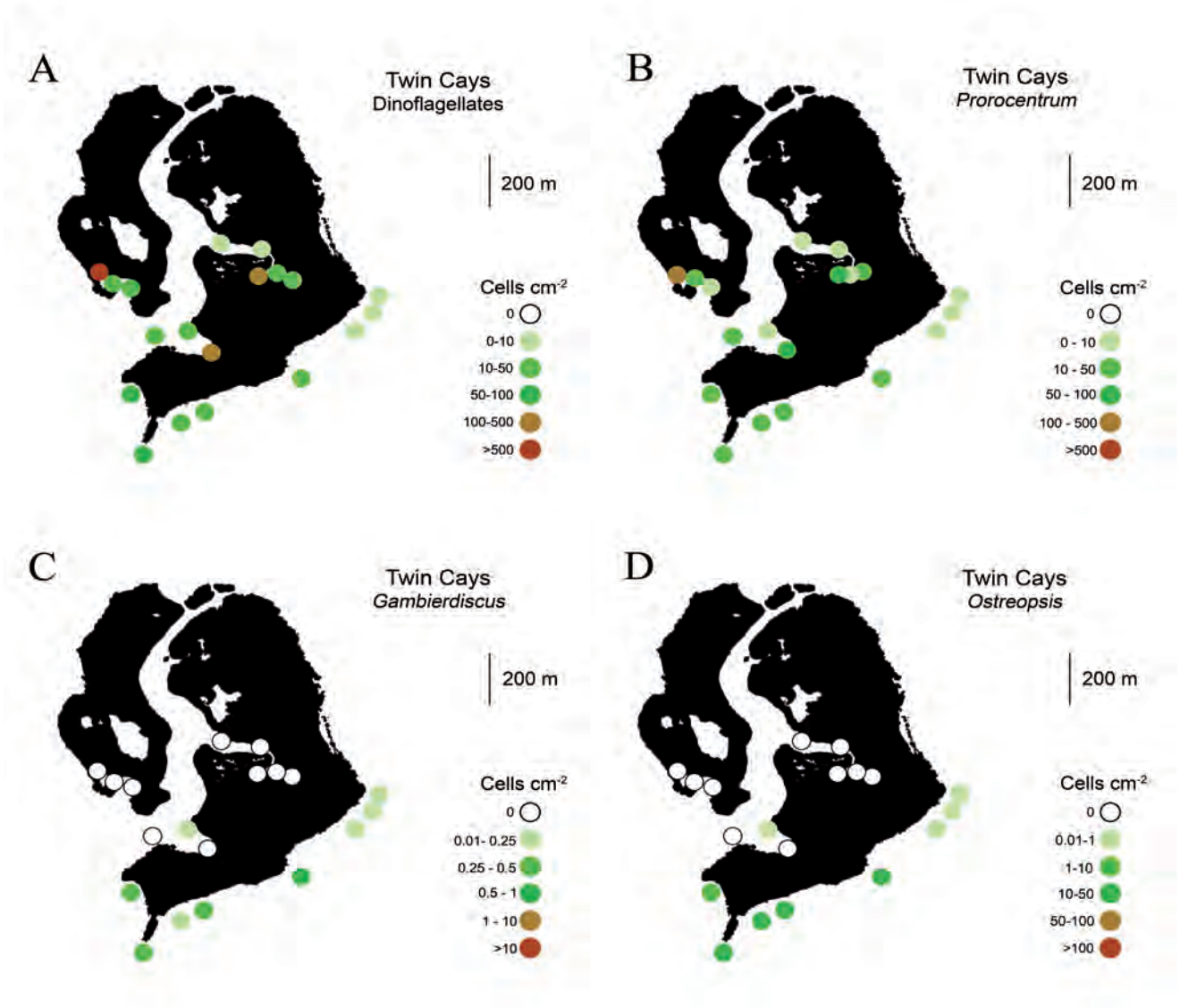


Figure 26. Distribution and abundance of (A) all dinoflagellates, (B) Prorocentrum*, (C)* Gambierdiscus *and (D)* Ostreopsis *cells collected at Twin Cays, Belize, May 2008 on artificial habitat and reported in cells cm^{-2} screen surface (Kibler et al., 2008).*

lato "as the sites where the macroalgae were directly hit by waves". In "calm regimes", i.e. "where macroalgae were protected from the waves by the rock barriers", *Coolia monotis* outnumbered *Ostreopsis* sp. The two taxa exhibited similar abundances at "intermediate conditions", so referred to as "slightly shaken". The results are similar to the observations by Richlen and Lobel (2011) at Johnston Atoll (Pacific Ocean) during the "calm summer months", where water motion is characterized using the "clod-cards technique". Although *Ostreopsis* spp. abundances were low in all habitats surveyed at Johnston Atoll, the relative numbers were higher at high energy reef crest/back reef sites and consistently lower in the lagoon/channel habitats. Carlson and Tindall (1985) also documented the highest abundances of *Ostreopsis* spp. in turbulent coral reef habitats. The resistance of *Ostreopsis* to high water motion has been attributed to its capacity to produce a mucilagenous matrix where cells aggregate and can attach to the macroalgae or other substrates. However, this capacity is also exhibited by other species such as *Gambierdiscus* (as indicated above) or *Prorocentrum* that prefer calmer habitats.

An illustration of the relative abundances of *Gambierdiscus* and *Ostreopsis* compared to *Prorocentrum* and all dinoflagellates collected on artificial substrates at a sampling site off Belize provides information on how small-scale habitat preferences of BHABs may vary (Figure 26). Kibler et al. (2008) found *Ostreopsis* 5-10 fold more abundant at high-energy sites compared to protected ones. The results were more variable from year to year for *Gambierdiscus* and they were not as readily assigned to a specific habitat type. *Prorocentrum*, *Scrippsiella* and *Protoperidinium* were consistently the dominant genera in protected mangrove habitats with limited water flow.

D4. Complex Interactions Among Physical and Biological Factors

Major questions remain concerning the extent to which the distribution and abundance of *Gambierdiscus* and *Ostreopsis* species depend on physical and chemical factors such as light, temperature, salinity and nutrients versus biological factors such as grazing. Benthic species are often in contact with sediments and macrophytes, which can supply nutrients. So it is not clear, whether nutrients play as large a role in controlling species abundances, as is the case for planktonic species. Similar questions also exist concerning how these same factors regulate the toxicity of species. The development of models for predicting likelihood of blooms and their relative toxicity will require more detailed information on the relative importance of physical and biological factors in controlling the abundance, distribution and toxicity of individual species. Studies designed to acquire these crucial data will be a major goal of the CRP.

E. Life Cycle and Ultrastructural Characteristics

E1. *Gambierdiscus*

In one of the few studies of *Gambierdiscus* life cycles, Hokama et al., (1996) observed *G. toxicus* at 200x and described them as motile cells, 45 µm diameter, with brown-colored pigments and relatively thin walls. As preparation for division, cells formed thick, translucent walls (2 µm thick) with dense, dark brown central cores that were interpreted as cysts. These cysts remained attached to the substrate; after gentle teasing, division occurred rapidly and produced cells with 0.5 µm thick cell walls and slightly brown inner pigments (Hokama et al., 1996). They followed up to 20 divisions per cyst. Hokama et al. (1996) provide a schematic asexual life cycle but there are no reports of differentially sized cells, gametes or a biflagellated planozygote indicating sexual reproduction. However, *Gambierdiscus* likely has a sexual life cycle. Taylor (1979) partially described and illustrated a planozygote and isogametes and Holmes (pers. Comm.) has unpublished photographs of what appears to be coupling gametes. **The opportunity to verify life cycles of this genus is an open invitation to new contributions to this CRP.**

Durand and Puiseux-Dao (1985) and Durand and Berkaloff (1985) examined the ultrastructure of *G. toxicus* and found the cytoplasm to contain various types of vacuoles, numerous trichocysts and a thick theca. They noted the photosynthetic apparatus was divided into two parts. Numerous thylakoids were visible in chloroplast sections and numerous vesicles appeared to contain nuclear material. *Gambierdiscus* has the standard peridinin containing chloroplast characterized by chlorophyll c2 and peridinin as major accessory pigments; chlorophyll c1 was detected as a minor pigment (chlorophyll c1/chlorophyll c2 = 0.12 - 0.17). Diadinoxanthin and dinoxanthin are also relevant pigments (Fraga et al., 2011; Zapata et al., 2012).

E2. *Ostreopsis*

Ostreopsis species have been reported to exhibit a relevant morphological diversity that was interpreted as indicative of different life stages, before systematic investigations on life cycles and transitions among these life stages were done. For instance, in post-exponential phase culture and in natural samples from Greek waters during the decline of the bloom two cell size classes were reported for both *Ostreopsis* cf. *ovata* and *O.* cf. *siamensis* (Aligizaki and Nikolaidis, 2006). The authors hypothesized that cells of a large size, would have derived from sexual fusion. In turn, non-motile, hyaline and rounded *Ostreopsis* cf. *ovata* morphs would constitute resting stages (Aligizaki and

Nikolaidis, 2006). Indeed, the presence of small cells in the life cycle is known for several dinoflagellates (Silva and Faust, 1995), where these cells have been interpreted as gametes (i.e. cells capable of conjugating and producing the diploid stage, planozygote). Smaller cells could also be produced under non-optimal nutrient concentrations.

Recently, a study by Bravo et al. (2012) on *O.* cf. *ovata* in cultures and natural populations isolated from Mediterranean water revealed that these small cells would not act as gametes as it had been suggested earlier. In contrast, the observed gamete pairs, mostly isogamous, were similar in size to the normal vegetative cells. Thus, the significance or the role of the small cells remains unsolved.

The main highlights of the investigation of the life cycle of *O.* cf. *ovata* by Bravo et al. (2012; Figure 27) were: a) it was not possible to distinguish zygotes from vegetative cells; b) mating gamete pairs were the only sexual stages that could be identified; c) no plasmogamy was observed although nuclear migration from one gamete to the other was observed; d) gamete pairs underwent division while they were still joint, although they did not encyst; e) the overwintering population of *O.* cf *ovata* in temperate waters could be constituted by pellicle cysts, thin-walled cysts and thecate cysts (able to germinate after 6 months in the laboratory).

Information on ultrastructure and pigment composition of *Ostreopsis* species is very scanty. An ultrastructural investigation on *O.* cf. *ovata* has confirmed that threads often seen in cultures and in natural samples are actually trichocysts (Honsell et al., 2010). Pigment composition of *Ostreopsis* is similar to *Gambierdiscus* with peridinin and chlorophyll c2 as main accessory pigments, but with the exception that chlorophyll c1 was not detected by HPLC in several strains of *O.* cf. *ovata* and *O.* cf. *siamensis* (Zapata et al., 2012).

By subjecting *O.* cf. *siamensis* cultures to low-nutrient stress, a great number of small cells, having fewer chloroplasts as compared to normal cells, were obtained in cultures from New Zealand waters (Chang, 1999). The fusion of these small cells gave rise to a non-motile zygote (somewhat smaller in size than normal cells) which developed a thick wall and showed a dark reddish-brown color and a granular content, with a high number of starch grains. Based on the presence of numerous thecal plates and empty thecae on the bottom of culture plates, asexual reproduction in *O.* cf. *siamensis* is reported to occur by discarding the theca (Chang, 1999).

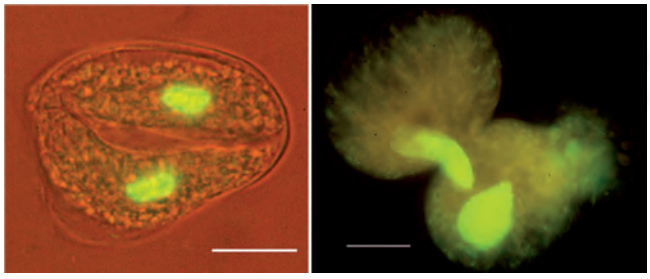


Figure 27. Light microscopy of mating cells (left) and an epifluorescent image showing vegetative cells undergoing cytokinesis (right). From Bravo et al. (2012), with permission from the editor.

F. Toxicity of *Gambierdiscus* and *Ostreopsis* species

F1. *Gambierdiscus*

Of the described species of *Gambierdiscus*, *G. polynesiensis*, *G. australes*, *G. belizeanus*, *G. pacificus* and *G. excentricus* are known to be toxic. Recently, Chinain et al. (2010b) tested the toxicity of four Pacific and one Atlantic species and found *G. polynesiensis* many times more toxic than any of the other species tested (Figure 28). According to the new definition of *G. toxicus* by Litaker et al., 2009, the only two properly identified strains (GTT-91 and REN-1) analyzed were non-toxic (Chinain et al., 2010b). This study used LC-MS and receptor binding assay methods to provide information on the 100- to 250-fold differences in toxicity among different species. Only limited data on the within-species toxicity differences are available, but it appears to be in the two-fold range. A survey of environmental effects on growth and toxin production indicated that changes in growth conditions caused approximately a 2- to 9-fold variation in toxin content per cell. These observations suggest that CFP events are driven more by inherent differences in species toxicity than by environmental modulation (e.g. Holmes et al., 1991; Litaker et al., 2010). Given the paucity of available data, however, this conclusion needs to be more rigorously evaluated. **Consequently, it is expected that**

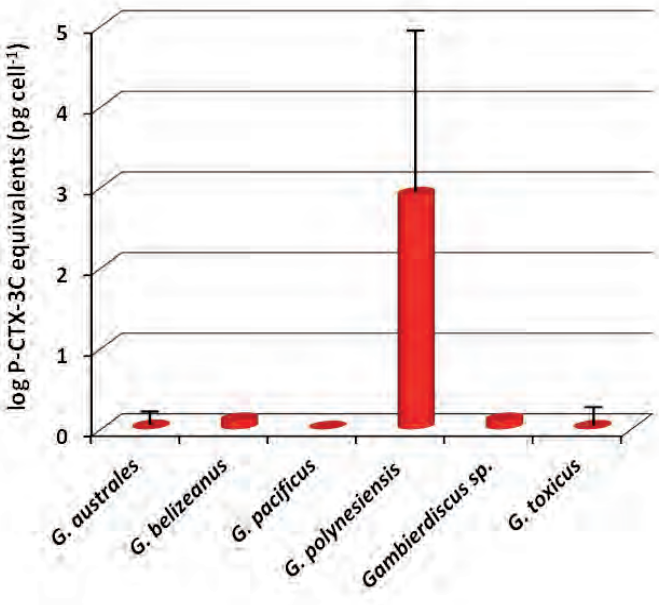


Figure 28. Variations in ciguatoxins-3C equivalents per cell for five Gambierdiscus *species (drawing from data published in Chinain et al., 2010b).*

the effects of environmental factors, in conjunction with determining intra versus interspecific differences in toxicity, will be an active area of research in in this CRP.

F2. *Ostreopsis*

Six *Ostreopsis* species have been reported as responsible for toxic events. Of these, three have been shown to produce potent toxin analogues of palytoxin, which are complex, high molecular weight, and water-soluble polyalcohols (e.g. Rhodes et al., 2002; Penna et al., 2005; Riobó and Franco, 2011). Ballantine et al. (1988) detected the presence of toxicity on extracts from *O. lenticularis* populations in outbreaks occurring in Puerto Rico. The production of ostreocyn-D was detected in *O.* cf. *siamensis* (Usami et al., 1995; Ukena et al., 2002), and mascarenotoxin-a and -b were found in *O. mascarenensis* (Lenoir et al., 2004). After the discovery of ovatoxin-*a* in Mediterranean strains of *O.* cf. *ovata* (Ciminiello et al., 2006), five more ovatoxin types (Ciminiello et al., 2008; Guerrini, 2010; Ciminiello et al., 2010, Rossi et al., 2010; Ciminiello et al. 2012) and two mascarenotoxins (Rossi et al., 2010) were detected for this species. The extent to which production of these toxins is controlled by environmental versus inherent genetic differences among strains and species has not been determined yet (e.g. Figure 29). Among the eight *Ostreopsis* clades differentiated by Sato et al. (2011) using genetic markers (which could correspond to several putative species), seven of them were toxic.

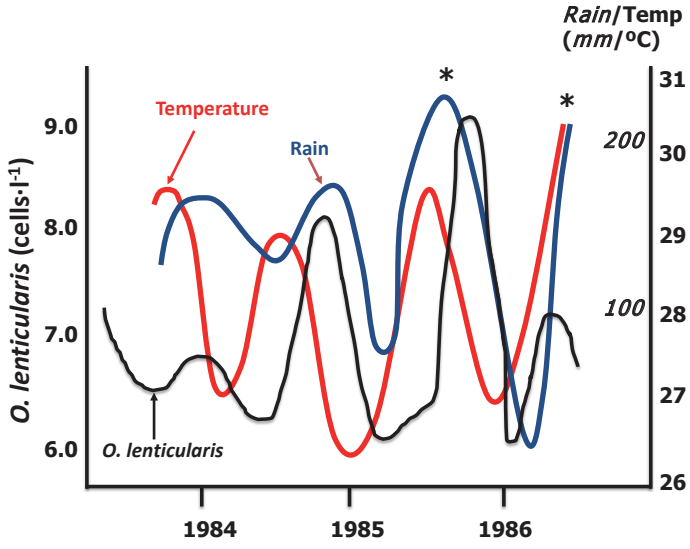


Figure 29. Seasonality in O. lenticularis *abundance (continuous black line) and toxicity (*) was documented in this time series from Puerto Rico. However, no direct link to environmental factors (temperature and rain) was established. Redrawn from Ballantine et al. (1988); left Y-axis: cell abundances in logarithmic scale; right Y-axis: double axis for rain (mm) and temperature (ºC).*

G. Effects of BHABs on Human Health

G1. Food Intoxications

The main toxins associated with BHAB species are gambiertoxin/ciguatoxins (GTX/CTX), maitotoxin (MTX) and palytoxin (PLTX) or its various analogs including ostreocin, mascarenotoxins and ovatoxins. These nonproteinaceous polyketides are among the most potent marine toxins. CTXs, GTXs and MTXs are produced by species in the genus *Gambierdiscus* (Yasumoto et al., 1977; 1980; Bagnis et al., 1985; Yasumoto, 2001; Chinain et al., 2010b). Originally it was believed that *Gambierdiscus* species produced GTXs, which were biotransformed to the CTXs that accumulate in fish. Chinain et al. (2010a, b) recently documented that many of the CTXs found in fish are actually produced directly by *Gambierdiscus* species. It is likely that the CTX in fish is the result of both accumulation of CTXs and biotransformation of GTXs. "Cigua" toxins comes from the Spanish for "one poisoned from snails" (cigua or sigua) and is attributed to sailors who became ill in the Caribbean. CTXs are both heat and cold stable. PLTXs were originally described from the zoanthid *Palythoa toxica* in the Hawaiian Islands (Moore and Scheuer, 1971), but have subsequently been isolated from species of the genus *Ostreopsis*, along with several analogues (Rossi et al., 2010; Ciminiello et al., 2011). These toxins travel through the food web and can contaminate fish (GTX, CTX, PLTX), crustaceans or molluscs (PLTX). It must be noted though, that although a palytoxin analogue was detected in the New Zealand strain of *O.* cf. *siamensis* (Rhodes et al., 2002), the risk to human health through consumption of shellfish contaminated by this species is unknown and further research is necessary to ascertain the potential risks (see e.g. Aligizaki et al., 2011, for a recent revision).

PLTXs and CTXs/GTXs are neurotoxins that act as sodium agonists in the synaptic channels (Lombet et al., 1987). The subsequent depolarization of membranes can sequentially cause paralysis, heart contraction, and changing the senses of hearing and temperature sensitivity. MTXs alter calcium channels (Yasumoto et al., 1976), with resulting cell death. The major symptoms of CFP develop within a few hours of toxin ingestion: vomiting; diarrhea; numbness of extremities, mouth and lips; reversal of hot and cold sensation; and muscle and joint aches. The symptoms may last from days to weeks, or even months, depending on each individual situation.

Short and long term debilitating gastrointestinal and neurological symptoms associated with PLTX poisoning include malaise and weakness, myalgia and impairment of the neuromuscular apparatus, abnormalities in cardiac function (e.g. Bagnis et al, 1979; Racciatti et

al., 2001; Taniyama et al., 2003). Cases of fatal intoxication ascribed to PLTX poisoning in subtropical and tropical waters have been associated with the ingestion of several different fish (*Herklotsichthys quadrimaculatus*, *Scarus ovifrons*, *Epinephelusbruneus* spp., *E. fuscogattus*, etc.) and crab (*Demania reynaudii*, *Lophozozymus pictor*) species (see Tubaro et al., 2011 for a review). However, in many cases the toxin identification is either incomplete or missing and the attribution of the poisoning to PLTXs, is only based on symptoms, anamnesis and ingestion of particular seafood.

Ciguatera fish poisoning, which is caused by the bioaccumulation of CTXs, is of significant concern in many tropical areas, where it has been known for centuries. Although mortality from ciguatera is low, morbidity is high and symptoms may be debilitating and prolonged. Common symptoms include diarrhea, vomiting, abdominal pain, reversal of temperature sensation, muscular aches, dizziness, anxiety, sweating, numbness and tingling of the mouth and digits, altered sense of smell, irregular heartbeat, reduced blood pressure, paralysis and rarely death (Friedman et al., 2008). Symptoms experienced by a given individual can be quite diverse and appear dependent on a combination of the amount of toxin consumed, the suite of toxins present in the tainted fish and an individual's susceptibility. Though the symptoms are relatively well documented, the disease often goes unreported or misdiagnosed.

Earlier studies reported the highest incidence of CFP in the world in the South Pacific, where the consumption of seafood is over 200 kg/capita/annum (Rapaport, 1999). Namely, 3400 to 4700 cases were recorded per year, although this value likely represented only 10-20% of the actual number of cases (Laurent et al., 2008). The incidence of ciguatera in each country ranged from 1/1,000-20/1,000 (Laurent et al., 2008). This trend may have been increasing in the last years, as revealed by the recent study performed by the Health and Fisheries authorities of the Pacific Island Countries and Territories (PICTs) in the Oceanica region. The reported cases (33,284) from 17 PICTs (Cook Islands, Fiji, French Polynesia, Guam, State of Hawaii, Kiribati, Marshall Islands, Nauru, New Caledonia, Niue, Northern Mariana Islands, Palau, Samoa, Tokelau, Tonga, Tuvalu, Vanuatu and Wallis and Futuna) showed that the mean annual incidence of ciguatera across the region for the 11 years from 1998 to 2008, was 229 people per 100,000 population. This proportion is more than twice the reported annual incidence of ciguatera for the 11 years from 1973 to 1983, which was 97/100,000. If the official reported CFP represents 20 percent of actual incidence (conservative estimate for food-borne illnesses) then the actual number of cases for this period could be as high as 166,420. It was estimated that, since 1973, approximately 500,000 PICT inhabitants have had CFP. Given the current population of the region surveyed, not including the State of Hawaii, is approximately 2,084,000, this means that approximately one in every four persons in the Oceanica region has had the disease. This level of incidence can place increased burden on coastal communities and national health services (Chinain et al., 2010a; Rongo and van Woesik, 2011; Skinner et al., 2011; Azziz-Baumgartner et al., 2012).

The impact of ciguatera is wider than the food intoxication itself. The concern of local populations regarding ciguatera has resulted in ca. 71% of people no longer eating local reef fish, as showed by a study performed on Roratonga, Cook Islands (Hajkowicz and Okotai, 2005). The result has been an increased reliance on pelagic fish and a less healthy diet of imported canned fish and red meat. This new diet, combined with reduced levels of physical exercise, and associated to a reduction in inshore fishing activity is contributing to increase obesity trends noted amongst Pacific Islanders (SPC, 2002). Indirectly, ciguatera fish poisoning is an additional burden on health care systems in the Pacific Islands. Furthermore, CFP has an economic impact on Pacific Island countries. A survey of ciguatera-poisoned patients in French Polynesia found that losses in productive working days through sick leave amounted to US $1 million/year (Bagnis, 1992), equivalent to US $1.64 million/year in 2012. In addition, the live reef fish trade was severely impacted in the country of Kiribati when people in Hong Kong contracted ciguatera from fish allegedly imported from Kiribati. The incident resulted in a total closure of the trade in Kiribati and a loss in income for several fishermen (Laurent et al., 2005).

The highest rates of CFP in the Caribbean from 1996 through 2006 were reported from the Lesser Antilles, the easternmost part of the Caribbean where 34 and 59

*Figure 30. Barter, trade or sale of barracuda (*Sphyraena*) is prohibited throughout most of the Caribbean Sea and southern Florida due to the high risk of ciguatera fish poisoning associated with this top predator (courtesy of M. Toscano, Smithsonian Institution).*

cases per 10,000 population per year were reported in Antigua-Barbuda and Montserrat, respectively (Tester et al., 2009; 2010). There appears to have been an increase in CFP incidence for Antigua-Barbuda and Montserrat in the Lesser Antilles since 1981, when Bagnis as cited in Tosteson (1995) reported 6 and 42 cases per 10,000 population respectively, though no cases were reported at that time to the Caribbean Epidemiology Center in either jurisdiction (Tester el al., 2009). While it is generally agreed across the Caribbean, as in other endemic areas, that CFP has been underreported (Tosteson, 1995; Tester et al., 2010), there are other difficulties associated with establishing robust measures of incidence rates. There is variability in the extent to which the population or their medical providers can identify CFP or in the consistency of reporting CFP to public health authorities. Incidence rates can be affected by different regulations across the Caribbean that prohibit selling or trading large, commonly ciguatoxic reef fish like barracuda (Figures 30, 31). Ciguatera fish poisoning incidence rates also depend on the extent to which the population relies upon locally-caught seafood as their primary source of protein, the extent to which fishers communicate about areas where ciguatoxic fish have been caught, as well as other factors like mandatory or voluntary reporting requirements. Data from both the Pacific and Caribbean collated from the literature documented that high incidence rates (e.g., over 100 incidences per 10,000 population per year) occur in both the Pacific and Caribbean, albeit with high variability (Table 1 in Tester et al., 2010). There was no clear trend in the high incidence rates among islands in the Pacific compared to those in the Caribbean. High incidence rates, while widely reported, represent only a small subset of the data for either the Pacific or Caribbean. Most annual incidence rates from both regions fell well below the 100 incidences per 10,000 population per year. While average CFP incidences were 2-fold higher in the tropical Pacific compared to the Caribbean (Tester et al., 2010), this would seem to corroborate the common assumption that the tropical Pacific has higher CFP incidence rates. However, Tester et al. (2010) were not able to substantiate a statistical difference due the high variability of the data reported for CFP occurrences.

Spatially intensive studies provide informative examples of how CFP incidence rates are driven by both sociological and environmental factors. Tosteson (1995) cites two studies; both were done in St. Thomas, U.S. Virgin Islands at about the same time. The first was a telephone survey by Tacket reported in a U.S. Center for Disease Control and Prevention (CDC) report of households from 1975 to 1980. The CFP incidence rate in this study was 72.4 cases per 10,000 population per year. The second study reported 440 cases per 10,000 population per year (McMillan et al., 1980) and was the product of a direct household canvass. Tosteson (1995) explained the difference by pointing out the telephone survey excluded lower income households that represented ‘‘regular consumers of seafood caught in the immediate area’’. A similar study by Azziz-Baumgartner et al. (who conducted a door-to-door survey of households in Culebra, Puerto Rico, in 2005–2006 and found the annual incidence rates of CFP on this small island were between 73.6 and 169.5 per 10,000 of the island’s population. Fewer than 10% of these cases were reported even though Puerto Rico has a mandatory CFP reporting requirement. Elsewhere in the Caribbean, it is noteworthy that CFP has in Trinidad and Tobago only within the last decade. In his study of the Caribbean, Bagnis (as cited in Tosteson, 1995) did not report CFP in Trinidad-Tobago in 1981. There were no CFP cases reported there until 1999, however, from 2000–2006 cases were reported to the Caribbean Epidemiological Center (http://www.carec.org, Tester et al., 2009).

In the Caribbean, Tester et al. (2009) found that CFP incidence data were not collected uniformly or consistently by country or territorial governments. Another interesting finding of their study was the wide range in the levels of concern and knowledge among Caribbean populations and the extent to which CFP is monitored

Figure 31. Ciguatera fish poisoning is a concern for subsistence fishermen in regions where alternative sources of protein are scarce (NOAA).

there. Some government agencies mandate central reporting of CFP cases, while other agencies acknowledged potential problems, but had been hampered by insufficient resources to initiate a monitoring system. Some health departments reported making progress toward bringing CFP surveillance programs online, sometimes in response to recent outbreaks of CFP. A few agencies asserted that CFP did not occur in their jurisdictions, an assertion that was not always consistent with the findings of the literature. This underscores the need for public health and fisheries professionals to receive up to date information about regional CFP occurrences. The absence of a uniform reporting procedure for CFP cases has hampered regional understanding of the scope of this important public health issue. **Future efforts of this CRP should support providing environmental monitoring data in tandem with bloom reports in formats accessible to resource managers and public health officials.**

G2. Skin and Respiratory Diseases

The presence of PLTXs and/or its analogues in seawater and in marine aerosols seems to be associated with skin and respiratory irritation (Botana, 2008). A series of case reports and anecdotal references describe poisonings after inhalation and cutaneous/systemic exposures after direct contact with aerosolized seawater during *Ostreopsis* blooms and/or through maintaining aquaria containing cnidarian zoanthids (reviewed in Tubaro et al., 2011).

Skin irritations and respiratory diseases (rhinorrea, cough) accompanied in some cases with headache, myalgy and/or fever have been experienced in tourist beaches in the Mediterranean (e.g. Bottalico et al., 2002; Sansoni et al., 2003; Álvarez et al., 2005; Masó et al., 2005; Gallitelli et al., 2005; Ciminiello et al., 2006; Squarcione 2007; Vila et al., 2008; Mangialajo et al., 2010; Illoul et al., 2012) and Brazilian coasts (Proença et al., 2010). A PLTX-like molecule, later described as ovatoxin-a, was identified as the major toxin produced by *Ostreopsis* cf. *ovata* (Ciminiello et al., 2008), while palytoxin like substances were found in plankton samples collected in the summers 2005 and 2006 along the coasts of the Ligurian Sea (Mediterranean Sea; Ciminiello et al., 2006), when up to 200 people were hospitalized presenting cough, dyspnoea, sore throat, rhinorrhea, fever, headache, lacrimation, nausea/vomiting and dermatitis (Durando et al., 2007).

Interestingly, since those years intense *Ostreopsis* blooms have occurred in the area in summer, without any report of similar syndromes (Tubaro et al., 2011). Indeed, despite recurrent summer and early autumn proliferations of *Ostreopsis* all over the rocky shores of Italy, France, Greece and Spain, the number of reports of effects ascribed to the dinoflagellate on human health were rather limited (Brescianini et al., 2006; Durando et al., 2007). In many cases, symptoms may not be severe and may go unreported. In fact, so far there was no demonstration of PLTXs in the aerosol during *Ostreopsis* blooms, or that the symptoms occasionally reported were actually caused by PLTXs. Similar reports of harmful effects on human populations were provided for other sites along the Italian coasts (Sansoni et al., 2003; Gallitelli et al., 2005), Spain (Barroso-Garcia et al., 2008), France (Kermarec et al., 2008; Tichadou et al., 2010), but in all places they were only irregularly linked to the presence or abundance of *O.* cf. *ovata* blooms in seawater. For instance, Proença et al. (2010) reported that extracts from scum and microperiphyton were toxic to sea-urchin larvae and produced haemolysis. Further, palytoxin-like peak with mass spectrum characteristic for ovatoxin-a, were detected (using LCMS/MS) on extracts from *O.* cf. *ovata*. However, no evidences of palytoxin were found in the tested aerosols.

Up to now, **it has not been possible to confirm the hypothesis that microalgae are the source of these intoxications.** This lack of evidence points to complex biological and environmental interactions. The likely scenario that could cause the observed illnesses is the co-occurrence of intense blooms in calm waters followed by persistent on shore winds. This example highlights the **need to investigate how coastal oceanographic processes may modulate the dynamics of BHABS.**

H. Ecological Consequences of BHABs

H1. *Gambierdiscus*

To date, *Gambierdiscus* research has focused on the adverse impact CTXs have on human populations from both a public health and fisheries perspective. Any adverse ecological effects caused by these organisms have not been investigated.

H2. *Ostreopsis*

According to Landsberg (2002) the effects of harmful algae on other organisms and ecosystem dynamics are largely unknown. However, some more recent studies have noticed the existence of such alterations concurrently to *Ostreopsis* outbreaks (see Ramos and Vasconcelos, 2010 for a review). Mortality of a variety of marine organisms and in particular, of sea urchins, during blooms of *O.* cf. *ovata* were reported by Granéli et al. (2002) in Brazil and by Sansoni (2003) and Vila et al. (2012) in the Mediterranean. Similarly, in northern New Zealand declines in populations of sea urchins (Shears and Babcock, 2003) coincided with blooms of *O. siamensis* during the 1999/2000 austral summer (N.T. Shears, pers. obs.). Given the importance of those echinoderms as herbivores (Steneck et al., 2002), their dynamics could affect that of the whole benthic

ecosystem as well. Although a direct link could not be established, *O. siamensis* populations were highly visible, exhibiting a distinctive rusty-brown colored mucilaginous film covering the reef, macroalgae and other sedentary organisms. More recently, an unprecedented bloom of *O. siamensis* occurred on shallow reefs in northern New Zealand in 2004, following a period of calm sea conditions with warmer than average water temperatures (Shears and Ross, 2009). The cover of *O. siamensis* was highly ephemeral and strongly related to temporal and spatial variation in wave action. Blooms were most prevalent at sites protected from prevailing swells where *O. siamensis* covered 30–60% of the reef with the concentrations on macroalgae reaching 1.4 x 10^6 cells g^{-1} wet weight, some of the highest concentrations recorded worldwide. Surveys of the health of sea urchins (*Evechinus chloroticus*) in relation to the cover of *O. siamensis* suggested strong negative effects on this ecologically important herbivore and urchin densities declined by 56–60% at bloom sites over the study period. In contrast, *Gambierdiscus* species have not been found to cause significant environmental damage.

Given the high spatial variability and ephemeral nature of HABs, their effects on marine organisms may go unnoticed and it is often difficult to attribute changes in marine communities to such events (Shumway et al., 2003). Furthermore, other environmental factors associated with HABs such as elevated water temperatures and depleted oxygen levels can also impact marine communities making it difficult to determine the actual mechanisms responsible for mortality events associated with HABs. Further research is needed, however, to determine the underlying direct (toxins) or indirect (e.g., wave action) mechanisms of action by *Ostreopsis* cells on sea urchins biology.

IV. KEY QUESTIONS TO BE ADDRESSED IN UNDERSTANDING THE ECOLOGY AND OCEANOGRAPHY OF HABs IN BENTHIC SYSTEMS

During the OSM in Honolulu, a long list of questions was compiled concerning unknowns about BHABs, which were lively discussed among participants. These questions have been grouped to define the key areas of research of the BHAB CRP following the structure of the five Program Elements of GEOHAB (Science Plan, GEOHAB 2001).

PROGRAMME ELEMENT 1: BIODIVERSITY AND BIOGEOGRAPHY

Overall Objective: To determine the relationships among distributions of BHAB species, biodiversity, and environmental change.

In many cases morphological characters do not seem to be sufficient to assess with certainty the identity of some benthic harmful dinoflagellate species of the genera *Gambierdiscus* and *Ostreopsis*. Therefore, the distribution range of the species of these genera is still to be ascertained at both large geographic and local scale. Specific objectives are to:

1. Improve the taxonomical knowledge on the *Gambierdiscus* and *Ostreopsis* genera, by combining both morphological and adequate genetic characterization.
2. Assess the genetic variability of *Gambierdiscus* and *Ostreopsis* species in relation to their toxicity, population dynamics and biogeography.
3. Determine the changes in the biogeographical range of *Gambierdiscus* and *Ostreopsis* species caused by natural and/or anthropogenic mechanisms. Studies will elucidate the environmental factors contributing to mesoscale distribution of these taxa, including possible modulations linked to climate change (ocean acidification, global warming). Research will also consider large scale dispersion of BHAB species mediated by human activities.
4. At small scale, investigate the relevance of substrate specificity of *Gambierdiscus* and *Ostreopsis*.
5. Investigate the possible role of other benthic taxa (e.g. *Coolia*, *Prorocentrum*, benthic diatoms and cyanobacteria) in BHABs.

PROGRAMME ELEMENT 2: NUTRIENTS AND EUTROPHICATION

Overall Objective: To determine the significance of eutrophication and nutrient transformation pathways to HAB population dynamics.

There is no clear direct link between eutrophication and BHABs, although this could be because knowledge of the nutrient requirements of *Gambierdiscus* and *Ostreopsis* species is still limited. Specific objectives are to:

1. Investigate whether nutrient inputs could indirectly affect BHAB outbreaks through an overall modulation of the whole phytoplankton assemblage and/or through the development of their necessary macroalgae substrates.
2. Investigate the mixotrophic capacity of *Gambierdiscus* and *Ostreopsis* species. Eventually, identify potential preys, estimate growth rates under mixotrophic conditions, and compare with autotrophic growth with inorganic and organic nutrient supply.
3. Investigate the possible nutritional links between BHAB species and their macroalgal substrate, such as sources of ,e.g., dissolved organic compounds.
4. Determine the effects of varying nutrient inputs on the harmful properties of BHABs, namely, toxin production and, possibly, the expression of toxicity-related genes.
5. Consider the potential role of groundwater discharges in BHABs.

PROGRAMME ELEMENT 3: ADAPTIVE STRATEGIES

Overall Objective: To define the particular characteristics and adaptations of BHAB species that determine when and where they occur and produce harmful effects.

BHABs are a particular case of noxious events, caused by organisms that may have intrinsic adaptive strategies sharing some benthic and planktonic charac-

teristics. Thus, understanding their intrinsic adaptive strategies may provide understanding of the initiation and persistence of those particular outbreaks that are not necessarily associated to high cell densities. Specific objectives are to:

1. Define the ecophysiological characteristics of BHAB species that determine their intrinsic potential for growth and persistence. Research should include adaptations to fluctuating light and temperature intensities; induction of resting stages, if any, and if so, their role in the population outbreaks; the existence of endogenous clocks versus external triggers to favor cell proliferation.
2. Investigate biological-physical interactions at small, local scale of the benthic assemblages and their substrate and microenvironment (including wave exposure, exposure/submergence, oxygen supply).
3. Describe and quantify chemical and biological processes affecting species interactions, including the role of mucous production, the potential role of toxins on marine animals at lethal and sublethal levels, parasite and viral infections, allelochemistry, competition and/or succession with diatoms and chemical interactions with the substrate.
4. Study the trophic transfer processes (fish, shellfish and other potential vectors grazing on macroalgae), which may affect human health.
5. Investigate how anthropogenic driven changes in the ecosystem structure (jellyfish outbreaks, fishing pressure, coral reef destruction) may affect trophic dynamics that in turn can alter benthic microalgal assemblages.

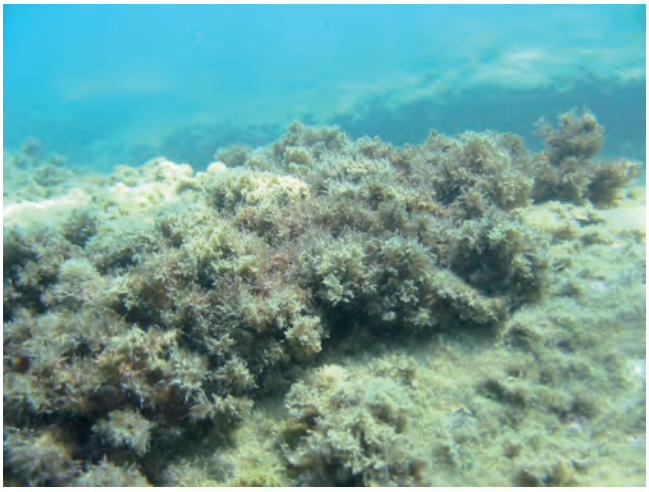
PROGRAMME ELEMENT 4: COMPARATIVE ECOSYSTEMS

Overall Objective: To identify mechanisms underlying BHAB population and community dynamics across ecosystem types through comparative studies.

Interestingly, *Gambierdiscus* and *Ostreopsis* are not typically found in the other systems covered under other GEOHAB CRPs (upwelling, stratified, eutrophied). The comparative approach can help to ascertain the environmental characteristics that favor BHABs. Possible comparisons would include:

1. Quantifying the occurrence of BHAB species in natural ecosystems with different characteristics, such as carbonate content, mangrove or seagrass or macroalgae communities, limestone vs volcanic islands, pebbles vs sandy substrates.
2. Address the link between fish size and CFP, which appears to be associated with large fish in the Caribbean but variable fish size in the Pacific. Why is it that only piscivores tend to be toxic in the Caribbean whereas both piscivores and herbivores are found to be toxic in the Pacific? Which toxic dinoflagellates are affecting corallivores? Do particular dinoflagellates have a preference for coral polyps as a habitat/host? Why is there no fish poisoning found on certain islands e.g. Easter Island? Does it have something to do with the macroalgae assemblages found there?
3. Investigate the role of coastal oceanography in bloom formation. Identify and quantify the effects of physical processes on accumulation and transport of benthic harmful algae in areas with different degrees of confinement. Consider in particular, the covariation of depth with light quality and intensity in the organisms' distribution.
4. Define functional groups in the benthic communities containing HAB species. Evaluate the similarities and differences among the communities found in the particular areas (Caribbean, Pacific, Mediterranean).
5. Compare the impact mechanisms between toxic aerosol-producing species of the genus *Ostreopsis* and *Karenia brevis*. A research on aerosol production is needed, considering e.g. investigation of the physical mechanisms that lead to aerosolization of toxins, compare the relative importance of bloom phases and meteorological conditions as drivers of aerosol formation.

To move forward internationally with a comparative approach, major efforts need to be done to establish a standardized sampling protocol. While the need to harmonize collection methods for benthic dinoflagellates has long been recognized, the techniques for doing so are diverse and challenging.



Macroalgae colonized by Ostreopsis *spp. in the NW Mediterranean (A. Zingone).*

PROGRAMME ELEMENT 5: OBSERVATION, MODELLING AND PREDICTION

Overall Objective: To improve the detection and prediction of BHABs by developing capabilities in observation and modeling.

At present we have poorly developed capabilities to observe BHAB organisms in situ, their properties, and the processes that influence them. In contrast to planktonic microalgae addressed in other CRPs within GEOHAB, benthic HABs are less amenable to numerical modeling approaches commonly employed in oceanography due to the complex interaction of toxic organisms, biological hosts, inert substrates, and the intricacies of benthic-pelagic coupling. Despite these limitations, simple models incorporating algal physiology and environmental conditions have been effective at predicting CFP (Parsons et al. 2010). BHABs could also be amenable to non-traditional (for oceanography) modeling approaches more commonly used in terrestrial environments, such as geo-spatial ecological approaches comparing (for example) the influence of island geological structure on BHAB prevalence. Because BHABs are also at least partially constrained by the availability of substrate and exhibit specific ecophysiological requirements (see Section D), BHABs may be an excellent candidate for combining climate change predictions with anticipated range expansion/contraction.

In addition, before substantial progress can be made regarding modeling of BHABs events, a major effort should be undertaken to accurately determine the abundance of benthic dinoflagellates. New and innovative methodologies should be developed to identify and estimate different *Gambierdiscus* and *Ostreopsis* species in natural samples collected during monitoring and/or survey activities. It is also important to identify which morphospecies and genotypes are associated to the production of palytoxin-like compounds. The community will be called upon to develop standardized sampling and identification protocols and use those in statistically robust studies.

V. FRAMEWORK ACTIVITIES

According to the GEOHAB Implementation Plan (2001), Framework Activities are those activities that are not research, but will facilitate the implementation of GEOHAB. They serve to enhance the value of the research by ensuring consistency, collaboration, and communication among researchers.

V1. Scientific Networking and Coordination of Resources

Scientific networking will support BHAB research. **Endorsement by GEOHAB** can improve the visibility (through the GEOHAB webpage) of BHAB-related projects and will facilitate coordination among different scientific teams that are currently working in this research field. At the OSM, some ongoing (or recently finished) projects were identified and are listed below. This list should be taken as indicative, because the elaboration of a complete one was outside the scope of this Report:

- New Zealand Seafood safety program, Cawthron Institute: it includes former protectorates of Cook Islands (coordination by Dr. L. Rhodes).
- University of Hawaii: the Ocean Health program includes a ciguatera research group; it has a fish focus (exotic grouper) liaising with groups for collection, structure of toxin, antibodies (coordination by Dr. P. Bienfang).
- Secretariat of Pacific Community (SPC) covering 22 PICTs by Fisheries Division: technical assistance, monitoring programs, sampling protocols, but no financial assistance. Some internally funded monitoring programs do exist in Marshall Islands, Samoa, Kiribati, Cooks Islands, Niue, French Polynesia.CFP data was once collected and now the collection has restarted. There has been an increase in requests from member nations for assistance in ciguatera management (see the conference held in Noumea in 2008). Current efforts are designed to develop a regional strategy to address issues and problems of member countries.
- New Caledonia: studies on cyanobacterial relations to ciguatera and the use of natural drugs and traditional remedies for CRP.
- Chile: no incidents of CFP but shellfish poisoning does occur. Easter Island (Rapa Nui) is also free of CFP; studying biodiversity of benthic HABs.
- French Polynesia (coordination Dr. M. Chinain) in collaboration with NOAA and other research groups assess outbreaks, taxonomy and toxin analysis.
- Vietnam, Institute of Oceanography: study of benthic HAB biodiversity and bigeography, monitoring about to begin; CLIMEET
- Malaysia (coordination by Prof. G. Usup): study of benthic biodiversity; use of RBA as monitoring tool; has had recent cases of CFP (Johor region).
- Indonesia (LIPI, Institute of Oceanography): BHAB monitoring; recent outbreaks in Thousand Isles (off Jakarta Bay) and Bali.
- Philippines (coordination by Prof. Azanza): isolated CFP outbreaks in the past; PSP monitoring program, a model for benthic HAB work.
- India (National Institute of Oceanography): studies of western side of subcontinent on HAB biodiversity; there have been coral bleaching incidents.
- Japan: studies on the CFP expansion, including toxin analysis and BHAB species biodiversity.
- Australia: CFP records being updated, ciguatera still occurring in the north and Great Barrier east coasts (coordination by Prof. R. Lewis and Dr. I. Stewart); working on food safety issues and bioassays.
- Italy: several Regional Environmental Agencies are very active in monitoring *O.* cf. *ovata* each summer, and the Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) organises a yearly coordination and information meeting where results of monitoring are shown along with news from science (e.g., see http://www.isprambiente.gov.it/site/it-IT/Pubblicazioni/Atti/Documenti/*ostreopsis*.html for the 2011 report). Different University groups are also involved in research projects funded by MIUR (Italian Ministery for Education, University and Research) on *Ostreopsis* ecology, genetic, physiology, toxin analytical chemistry, toxicological and biochemical pathway characterization.
- French National Health Agency in coordination with the MediOs project: BHABs monitoring and public health surveillance in the Mediterranean French coast.
- In Spain, the different monitoring programs on plankton HABs are including benthic organisms in their survey. The programs are conducted by the autonomous governments (Galicia, Andalucía, Murcia, País Valencià, Catalunya, Balears and Euskadi) in collaboration with research institutions (Spanish Research Council, Spanish Institute of Oceanography).
- In Portugal, Greece, Tunisia, Morocco, Algelia and Egypt, toxic outbreaks related to high abundances

of *Ostreopsis* are fostering increasing monitoring and research.

- In the US, the NOAA supported Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program is supporting CFP related projects in the Caribbean that focus on the ecology and toxicology of *Gambierdiscus*.

V2. Identification of Protocols and Quality Control

As in the case of planktonic HABs, GEOHAB encourages the use of existing (standard) protocols and guidelines for sampling and experimental methods. As already noted, there is an urgent need to establish uniform and standardized sampling procedures for BHABs.

To this end, one of the outcomes of the HABs in Benthic Systems workshop was to organize and facilitate a sampling workshop where methods can be compared and inform decisions on a standardized protocol for sampling benthic dinoflagellates. A workshop combining field exercise with taxonomic and molecular training was scheduled for June 2012 coordinated by Drs. G. Usup and P. A. Tester. A subsequent outcome from that workshop will be the establishment of an international data base platform.

V3. Capacity Building

Capacity building will ensure that a new generation of scientists is trained in the study of HABs. GEOHAB can meet its objectives only if well-trained scientists from a wide range of countries are involved. Within its endorsed projects, GEOHAB will encourage a "training through research" approach that offers opportunities for student participation in cruises and instruction in marine research disciplines relevant to HABs.

One way to encourage exchange is through the development of joint web resources such as the one contracted by Dr. Mona Hoppenrath (http://www.dinophyta.org/). Another example is the **Wikispaces** page (http://gambierdiscuswiki.wikispaces.com/) which has been created as a centralized location for information exchange and updates concerning *Gambierdiscus* species. The project arose from discussions held among scientists who attended the GEOHAB Workshop on Benthic Dinoflagellates, University of Hawaii at Manoa, 24-28 June 2010. The site is intended to be a forum for discussing the evolving taxonomy of *Gambierdiscus* species and inform and/or facilitate the following:

- Features and images of each described species
- A current list of known ribotypes which represent potentially undescribed species
- A means of obtaining a unique ribotype designation for a putative new species prior to formal description
- Useful information about PCR amplification and sequencing primers
- Updated *Gambierdiscus* species distributions
- Sampling protocols
- Culture methods
- Useful references

A new taxonomic book that will help foster BHAB research is in preparation by Hoppenrath and collaborators (expected publication in September 2013).

Some of the ongoing training programs include:

- NOAA's Center for Coastal Environmental Health and Biomolecular Research: training program for Latin American scientists to learn how to use the receptor binding assay for CTX at the NOAA lab in Charleston, South Carolina under the sponsorship of UNESCO and IAEA. Contact: Drs. Steve Morton or John Ramsdell. Spring 2012.
- NOAA's Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina: cross laboratory assay training with staff from Institut Louis Malardé for molecular identification and quantification of *Gambierdiscus* species using qPCR. Contact: Dr. Wayne Litaker. Spring 2012.
- NOAA's Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina: identification and culture of benthic HABs for staff from the University of the West Indies and Ministry of Agriculture and Fisheries, Jamaica. Spring 2012.
- Dr. Gires Usup, University Kebangsaan, Malaysia in collaboration with NOAA's Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina: training at students' workshop for molecular identification and quantification of *Ostreopsis* and *Gambierdiscus* species using PCR. Contact: Drs. G. Usup or Wayne Litaker. Summer 2012 and

Sampling for Ostreopsis *spp. in the Mediterranean (A. Zingone).*

winter 2012-2013.

- Dr. Gires Usup, University Kebangsaan, Malaysia in collaboration with D. Jacob Larsen, University of Copenhagen and Dr. Wayne Litaker from NOAA's Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina: phytoplankton identification workshop with an emphasis on BHABS. Summer 2012.
- Dr. Gires Usup, University Kebangsaan Malaysia in collaboration with NOAA's Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina: field training for sampling methods for BHAB species and validation exercise for artificial substrate as a way of estimating BHAB abundance. Contact: Drs. Gires Usup or Pat A. Tester. Summer 2012.

V4. Other activities

Aerosol studies. Another framework activity would include the study on the link between BHAB dynamics and respiratory diseases. It is still uncertain whether toxins have an aerosol transmission, and if so, how they are transported. This is a clear aspect where the impact on human health may be **strongly controlled by coastal oceanography**. The studies on the dynamics of BHABs should take into account oceanographic aspects, as it has been the case for harmful planktonic species.

Special session or meetings. In April 2011, a 1st International Conference on *Ostreopsis* Development (ICOD) was held in Villefranche-sur-mer (France). ICOD aimed to regroup the knowledge on 1) ecological, chemical and toxicological aspects of *Ostreopsis* species and 2) on the different methods of ecologic, economic and health management of the problem. The proceedings of the conference are available at:

http://www.cryptogamie.com/pagint_en/recherche/affich_sommaire.php?cnumero=79.

Similar aims can be addressed on *Gambierdiscus* at the 16th ICHA, Wellington, New Zealand (fall 2014). A special session is being organized by Dr. P.A. Tester to allow international exchange of information on projects that address BHAB core research projects.

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APPENDIX I – Open Science Meeting Program

DETAILED PROGRAMME

Notes for Participants

Talks: Invited speakers will be expected to adhere to the allocated times for their talks. The chairs of the plenary sessions will be strict about the timing, in order to keep the conference running smoothly.

Posters: Posters can be set up from 8:00 to 9:00 a.m. on Monday, 21 June. Supplies will be provided for mounting the posters. Posters will be left up for the entire meeting to make it possible for participants to view them during coffee breaks and lunch times. Posters should be taken down in the afternoon of Wednesday, 23 June.

Program

June 21 (Monday) (Hawaii 1 and 2)

Session Chair: Mona Hoppenrath, Senckenberg Research Institute, Wilhelmshaven, Germany (30 minute presentations and 15 minutes for questions and answers)

8:00 a.m. Poster Set up and Continental Breakfast

9:00 a.m. Introduction to GEOHAB – Raphe Kudela, University of California at Santa Cruz

9:30 a.m. Introduction to Open Science Meeting – Paul Bienfang, Convener, University of Hawaii

9:45 a.m. *Gambierdiscus* taxonomy --morphology & DNA methods – Wayne Litaker, NOAA Beaufort Lab, USA

10:30 a.m. Break

11:00 a.m. Morphological Identification and Taxonomy of Potentially Toxic Benthic Dinoflagellates – Katarina Aligizaki, Aristotle University of Thessaloniki, Greece

11:45 a.m. Genetic Diversity of Three Harmful Benthic Genera of *Ostreopsis*, *Coolia* and *Prorocentrum* – Antonella Penna, University of Urbino, Italy

12:30 p.m. Sampling Methodology for Benthic Dinoflagellates – Patricia A. Tester and Steve Kibler – NOAA Beaufort Lab, USA

1:15 p.m. Lunch (Kauai Room)

2:15 p.m. Breakout Session

1. Review of current national and regional projects/programs to identify elements of research that could contribute to core research & identification of interested participants and designated regions for comparative research - Pacific/Asia Region (Hawaii I)

   Discussion Leader: TBD

   Rapporteur: TBD
2. Review of current national and regional projects/programs to identify elements of research that could contribute to core research & identification of interested participants and designated regions for comparative research - Mediterranean and Caribbean Seas (Hawaii II)

   Discussion Leader: TBD
   Rapporteur: TBD

3:30 p.m. Break

4:00 p.m. Poster Session (Hawaii 1 and 2)

6:00 p.m. Adjourn for the Day

June 22 (Tuesday)

Session Chair: Pat Tester, National Oceanic and Atmospheric Administration

8:00 a.m. Continental Breakfast

9:00 a.m. A simple model capable of simulating the population dynamics of *Gambierdiscus*, the benthic dinoflagellate responsible for ciguatera fish poisoning – Michael Parsons, Florida Gulf Coast University

9:45 a.m. A Review of the Ecological Factors Driving Biogeography of *Gambierdiscus* Species – Lesley Rhodes, Cawthron Institute, New Zealand

10:30 a.m. Break

11:00 p.m. Overview of the Distribution of *Ostreopsis* and Benthic *Prorocentrum* Species Around the World Seas – Adriana Zingone, Stazione Zoologica Anton Dohrn, Italy

11:45 p.m. Reports Back from Monday Breakout Sessions

12:30 p.m. Lunch

1:30 p.m. Breakout Sessions

1. Research priorities and approaches for *Gambierdiscus* (Hawaii I)

   Discussion Leader: Lesley Rhodes

   Rapporteur: Jacqui Evans

2. Research priorities and approaches for *Ostreopsis*, *Prorocentrum* and *Coolia* (Hawaii II)

   Discussion Leader: TBD
   Rapporteur: Adriana Zingone

June 23 (Wednesday)

Session Chair: Jacqui Evans, Te Ipukarea Society Inc.

8:00 a.m. Continental Breakfast

9:00 a.m. Ecophysiology of *Gambierdiscus* – Steve Morton, NOAA Charleston Lab, USA

9:45 a.m. Foodweb effects and/or controls of *Gambierdiscus* (i.e., top-down-bottom-up controls, grazing controls, foodweb toxicity, etc.) – Brian Lapointe, Harbor Branch Oceanographic Institution, USA

10:30 a.m. Break

11:00 a.m. Ecophysiology of *Ostreopsis* & *Prorocentrum* species (i.e., adaptive strategies, physiological characteristics, life cycle traits, temperature preferences & tolerances, growth rates) – Lam Nguyen, Institute of Oceanography, Vietnam

11:45 a.m. What is Different in Benthic vs. Planktic HABs? – Santiago Fraga, Francisco Rodríguez, Isabel Bravo, and Manuel Zapata, Instituto Español de Oceanografía, Spain

12:30 p.m. Lunch

1:30 p.m. Report Back from Breakout Sessions

The ciguatera fish poisoning "plague" in the Pacific Island Countries & Territories, 1999 to 2008: results of a recent questionnaire. – Mark Skinner, University of Queensland

1-slide presentations of priority research topics by any participant

Identification of cross-cutting research issues and new topics following break-out group reports

3:00 p.m. Break

3:30 p.m. Next Steps and Timeline

4:00 p.m. Adjourn

6:00 p.m. Meeting Dinner at Tiki's Bar and Grill (2570 Kalakaua Ave.)

June 24 (Thursday)

Writing Session - Planning Committee only (University of Hawaii)

APPENDIX II – Meeting Participants

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